A STUDY OF ESTRUS SYNCHRONIZATION WITH PGF2α IN BRAHMAN HEIFERS: PROPOSAL OF A NEW SYSTEM

By

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... the barnyard was an expression of something that was real, vital, and fluid, that ... was of natural and spontaneous growth, that ... turned with its surroundings, that ... was a part of the life that offered itself to her.

Edith Summers Kelley
Weeds, 1923

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A STUDY OF ESTRUS SYNCHRONIZATION WITH PGF2α IN
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A study was conducted to examine the efficacy of a natural prostaglandin F2α (PGF2α) for synchronizing estrus in Brahman heifers. Estrous response rate and plasma progesterone (P4) concentrations in heifers treated with PGF2α were compared to determine whether induced corpora lutea (CL) produced lower concentrations of P4 than spontaneously occurring CL.

Fewer heifers expressed estrus within 7 d following a single PGF2α injection on d 7 of the estrous cycle than when injected on d 14 (61% vs. 100%, $P<.05$). Heifers injected on d 7, d 10, d 14, or d 18 demonstrated a graduated response rate (50%, 67%, 100% and 100%, respectively). Importantly, PGF2α induced a normal functioning CL since plasma P4 profiles did not differ between induced and spontaneous estrous cycles. When plasma samples were collected from 1 d before injection to 3 d after estrus (or to 6 d after injection in non-responders), P4 concentrations decreased by 12 h after injection in all heifers. Although there was a precipitous decline in P4, heifers that failed to express estrus had P4 concentrations that began to increase within 48 h after injection and reached concentrations greater than in heifers exhibiting estrus ($P<.001$).

Two injections of $PGF2_\alpha$ administered at a 24 h interval induced estrus more effectively than a single injection (97% vs. 72%, $P<.02$). Heifers treated with two injections at a 24 h interval were more tightly synchronized than heifers given a single injection ($P<.06$), with 94% of the double injection heifers expressing estrus in a 36 h period from 2.0 to 3.5 d after the first injection.

Data indicate a decreased estrual response to $PGF2_\alpha$ when given early in the estrous cycle. Injection of $PGF2_\alpha$ on d 7 or d 10 initiates a decline in plasma P4 but fails to pecipitate complete luteolysis in all heifers. A system is proposed that uses a series of three injections (second injection given 11 d after the first and third given 24 h after the second) that would result in more animals in estrus and in a tighter synchrony necessary for artificial insemination by appointment.

INTRODUCTION

The American Brahman and other Zebu breeds of cattle play a vital role in the beef industry of tropical and subtropical areas of the world. Bos indicus cattle are exceptionally well adapted to the hot, humid climate that typifies the tropics. Their ability to flourish under conditions of heat extremes, insect infestations and endzootic disease make use of Zebu cattle in purebred and crossbred operations in the tropics highly desirable and, in many circumstances, essential.

One basic requirement of successful cattle production is continuous progress towards genetic improvement in the herd. The most expedient way to hasten genetic progress is to use artificial insemination (AI) to increase the proportion of cattle mated to superior sires. Naturally bred Brahmans are reported to have lower pregnancy rates when compared to Bos taurus breeds (Burns et al., 1959; Kincaid, 1962; Koger et al., 1973; Crockett et al., 1978). Brahman producers have reported the pregnancy rate to AI after prostaglandin $F2_\alpha$ ($PGF2_\alpha$) estrus synchronization is also generally lower than that expected in a Bos taurus herd. These reports were corroborated by Tucker et al. (1982) and Landivar et al. (1985), but Adeyemo et al. (1979) found no difference in pregnancy rates to AI between Bos taurus and Bos indicus cattle. Randel et al. (1984) reported pregnancy rate was higher in Brahman cows than in Brangus cows that had been synchronized with the $PGF2_\alpha$ analog, alfaprostol.

Results of synchronization attempts with a $PGF2_\alpha$ analog (cloprostenol) in Brahman-crossbred females have been inconclusive (Hardin et al., 1980a,b). Hardin

and Randel (1982) reported Brahman cows treated with cloprostenol on d 8 to 12 of the estrous cycle subsequently developed smaller corpora lutea (CL) with lower progesterone (P4) content than the CL of untreated cows. In addition, serum P4 was lower in Brahman cows from d 2 to 13 of a cloprostenol induced estrous cycle than in naturally occurring cycles. The authors suggested impaired development of the induced CL could be responsible for the lower fertility of Brahman and Brahman-type females and that formation of this sub-functional CL might be one factor in the poor estrus response to prostaglandin synchronization. More recently, Hansen et al. (1987b) reported the CL formed following regression induced by the $PGF2_\alpha$ analog alfaprostol had fewer large and small luteal cells (in heifers) and lower in vitro P4 production in response to LH when compared to the CL formed following spontaneous estrus (in heifers and cows).

The studies presented here were designed to 1) examine the efficacy of a natural $PGF2_\alpha$ in synchronizing estrus in Brahman heifers, and 2) to determine whether the $PGF2_\alpha$-induced CL produced lower concentrations of plasma P4 than spontaneously occurring CL in Brahman heifers.

REVIEW OF THE LITERATURE

Artificial Insemination, Estrus Synchronization, and the Role of the Corpus Luteum

Artificial insemination is the placement of spermatozoa in the female reproductive tract by artificial instead of natural means. According to legend, the first AI was perpetrated in 1322 when an Arab chieftain used artificial methods to breed a valuable mare with semen surreptitiously collected from the sheath of a stallion belonging to an enemy tribe (Ensminger, 1976). The first research in AI of animals was conducted with dogs in 1780 by the Italian scientist Lazarro Spallanzani (Foote, 1986). By the next century, American scientists were artificially inseminating mares that had failed to settle by natural service (Ensminger, 1976) and by 1907 the Russian scientist Ivanov was reporting success in the AI of mares, cows and ewes as a method for widespread genetic improvement in livestock (Foote, 1986). Ivanov developed procedures whereby semen was collected from the epididymides of slaughtered bulls, diluted, and used to AI cows. If the cows were some distance from the abattoir, the epididymides were refrigerated and spermatozoa were removed later for insemination. This technique allowed the AI of cows at a distance of up to 2 h travelling time (Willet, 1956).

AI of cattle was first performed in the United States in 1937 and 1938 at the Agricultural Experiment Station in Minnesota (Foote, 1986). Cattle producers were quick to recognize the advantages of AI in allowing them access to superior sires that previously were available exclusively to the owners. With the advent of technology for

preservation of semen by freezing, long-term storage and long-distance transport became routine. By 1982, two-thirds of the 11,000,000 dairy cows in the U.S. were AI, but only 4% of the beef cows (Foote, 1986).

While the benefits of AI are apparent, the implementation of a successful program requires superior management of reproduction in a herd. In dairies it is a relatively simple matter to AI the dairy cow at the proper time following detection of estrual behavior (receptiveness of the cow to sexual overtures of the bull). This system of observation and breeding is less readily accomplished in beef cattle herds. In general, beef cattle are observed only occasionally, and if an AI program is desired, special handling procedures and facilities must be established. The necessary detection of estrus is time-consuming, difficult, and subject to human error. Systems of estrus synchronization facilitate the prognostication of the time of estrus with a reasonable degree of accuracy. This minimizes the amount of time a cattle producer must invest in estrus detection and may, with some procedures, make it possible to AI at an appointed time regardless of the manifestation of estrus (Hafez, 1987). Artificial insemination is most successful when the time of estrus expression is regulated and the hour and date of insemination precisely determined.

Estrus synchronization systems are ways in which times of estrus and ovulation in a herd are regulated or "synchronized" so that all herd members are at the proper stage of the estrous cycle for insemination at one time. Not only does synchronization facilitate the AI program, but it produces the added benefit of more cows calving earlier in the calving season.

In the cattle industry, as in most other businesses, "time is money." To cattle producers this "time" is best measured by calving interval. Dairymen recognize that

milk per day of calving interval decreases as days open increase. This is because additional days open result in more days in milk, which extend the low-producing part of lactation, and in more days dry. Each additional day open results in 4.5 kg less milk from heifers and 8.6 kg less milk from cows (Olds et al., 1979). Barr (1975) determined that Ohio dairies increased calving interval by 14.7 d due to failure of the cow to conceive once inseminated, but added 40.3 d due to failure of the herdsman to notice estrus. Dairy cows and heifers treated with a PGF2α analog were AI sooner in the season and became pregnant sooner than untreated controls (Seguin et al., 1983). Estrus synchronization with PGF2α resulted in conception occurring 22 d earlier in treated dairy cows than in cows that were AI without synchronization (Plunkett et al., 1984). PGF2α-synchronized AI also reduced the interval to estrus after parturition in Holstein cows (Lucy et al., 1986).

The value of synchronization is also evident in beef herds. Lambert et al. (1976) reported fertility was greater in beef cows synchronized with PGF2α and that the system resulted in more cows conceiving early in the breeding season. In one study, beef cows were treated with 25 mg PGF2α on d 5 of the breeding season unless they had previously been seen in estrus on d 1 to 4 and subsequently AI. All treated cows were AI at the detected estrus until 80 h after injection at which time all remaining cows in the groups were AI. Cows in control groups were not synchronized but were AI at estrus throughout the breeding season. Estrus-synchronized cows conceived earlier in the season, as 45.5% of the PGF2α treated cows vs 26.1% of the control cows were pregnant to inseminations during the first 10 d of the breeding season (Higgins et al., 1981).

One early method for altering length of estrous cycle and thus controlling time of estrus was manual extirpation of the well-developed CL from the ovary. This resulted in 90% of treated cows expressing estrus within 2 to 4 d (Willet, 1956). There is some hazard associated with this method in that hemorrhage and(or) adhesions may occur and possibly result in permanent damage to the treated cow. This rather crude technique, however, results in the same outcome sought by hormonal controls, i.e. destruction of the CL. Estrus synchronization methods seek to precipitate a premature demise of the CL at a predetermined and synchronous time in a group of treated animals.

The timing of estrus in domestic livestock is regulated by the production of the steroid hormone progesterone (P4) from the CL (Hansel and Convey, 1983). Progesterone prepares the uterus for reception and growth of the embryo. If pregnancy does not occur the CL begins a natural regression at about d 17 of the estrous cycle (estrus = d 0). Regression of the CL is accompanied by declining P4 concentrations and within 1 to 5 d of the beginning of this drop in P4 estrus results and is followed by ovulation (Stabenfeldt et al., 1969).

Progesterone acts by employing a negative feedback control on luteinizing hormone (LH) from the hypothalamus. As long as P4 concentrations are elevated the pre-ovulatory surge of LH is suppressed. It is this LH surge that precipitates estrus and ovulation (Hafez, 1987). Thus estrus synchronization is the control of the existence of the CL (Hansel and Convey, 1983). An understanding of the processes by which estrus synchronization is realized requires knowledge of the manner in which natural luteolysis occurs.

Interrelationship of the Uterus and Ovary

Loeb (1923, 1927) was the first to document a link between the uterus and ovarian function in a series of classic experiments in which guinea pigs were hysterectomized. Removal of the uterus resulted in preservation and continued function of the CL. The length of extended diestrus following partial hysterectomy was inversely proportional to the amount of uterus remaining after surgery. He concluded the uterus was implicated in control of the demise of the CL in the guinea pig.

The uterus appears to exert the same regulating influence in domestic livestock. Wiltbank and Casida (1956) found that hysterectomy prolonged the life of the CL in ewes and cows. Hysterectomy in heifers resulted in retention of the CL for at least 270 d (Anderson et al., 1962). It was suggested that CL regression was dependent on stimulus from the uterus. In the sow, total hysterectomy before d 16 of the estrous cycle results in protracted diestrus (Spies et al., 1960; Anderson et al., 1963), but if the uterus is removed between d 16 and 18, estrus is usually expressed at the expected time. Dissimilarly, regression of the CL, estrus and subsequent ovulation are prevented by hysterectomy up to the last day of the cycle in ewes (Kiracofe et al., 1966). In the mare, removal of the uterus results in persistence of luteal activity (Stabenfeldt and Hughes, 1977), but functional luteolysis (decrease in P4) is often observed after 30 to 40 d (Ginther and First, 1971).

Destruction or damage of the uterine endometrium by corrosives (Anderson et al., 1961) or infection (Coudert and Short, 1966; Ginther, 1968) causes the CL to be maintained for extended periods. However, insertion of various intrauterine devices (IUDs) into the uterine lumen shortens estrous cycle length in cows (Anderson et al., 1965) and ewes (Ginther et al., 1966a). Distention of the uteri of cows with a sterile

gel results in premature estrus (Yamauchi et al., 1967). A plastic spiral coil placed in one uterine horn in ewes inhibited sperm transport and ovum fertilization on both sides of the tract. The effects of the IUD were therefore due to something other than mechanical interference (Hawk, 1970). It became evident that destruction or devastation of the endometrium prolonged CL lifespan, but irritation or distention resulted in shortened cycles. Clearly the uterus was providing a luteolytic agent.

Extirpation of the uterus in sheep results in prolonged life of the CL (Moor and Rowson, 1964). Unilateral hysterectomy, whether alone or in combination with unilateral ovariectomy, consistently prolonged life of the CL on the ipsilateral ovary, but failed to affect the CL on the contralateral ovary in the ewe (Inskeep and Butcher, 1966; Moor and Rowson, 1966) or in the heifer (Ginther et al., 1967). Insertion of a plastic spiral coil into one uterine horn of ewes caused CL regression on the ovary ipsilateral to the coil (Ginther et al., 1966a)

In related experiments, daily administration of oxytocin (OT) to cycling dairy heifers during the first week of the estrous cycle resulted in a shortened estrous cycle of 8 to 12 d (Armstrong and Hansel, 1959). Oxytocin causes premature expression of estrus in heifers that have the uterus intact or have removal of the uterine horn contralateral to the CL. This does not occur when OT is injected in heifers completely hysterectomized or in which the ipsilateral horn is unilaterally removed (Ginther et al., 1967; Brunner et al., 1969). These observations indicate luteolysis is somehow mediated by the uterus in a local fashion in these species.

Since the ovary with CL must be in close proximity to the uterus for luteal regression to occur, it would seem likely that surgical separation would result in prolonged luteal function in these species. In fact, Goding et al. (1967) and McCracken

et al. (1971) reported that when the ovary or uterus in the ewe was autotransplanted to the neck, this is precisely what happened. However, other researchers have reported long and irregular cycles in cows following this procedure (Hansel and Snook, 1970) or normal cycles when the uterus is transplanted to the omentum in ewes (Niswender et al., 1970). Hansel and Snook (1970) attributed the irregular cycles in the cow to removal of the ovary from local luteolytic influence of the uterus, but Niswender et al. (1970) suggested maintenance of the CL in animals with uterus and ovary separated may be an artifactual result of the surgical procedure. This seems unlikely as sham-operated animals continue to cycle normally (Wiltbank and Casida, 1956; Ginther et al., 1966b; Moor and Rowson, 1966; Lamond et al., 1973). In the study conducted by Niswender et al. (1970), ewes with the entire uterus transplanted to the omentum had a silicone rubber cannula placed in the cervical end of the uterus for drainage of uterine effluent into the abdominal cavity. The possibility of a local effect of a uterine luteolytic agent on the CL within this cavity can not be excluded. When both the ovary and uterus were transplanted to the neck of ewes, normal luteal function appeared to confirm that these organs must be contiguous for luteal regression and ovarian cyclicity (McCracken et al., 1971).

Other species seem to be able to effect luteolysis by other than local means. In the mare, the route by which the uterus exerts its influence on the CL appears to be systemic. Total hysterectomy of the mare results in prolonged maintenance of the CL but unilateral hysterectomy had no effect on cycle length and failed to indicate involvement of a direct utero-ovarian relationship (Douglas et al., 1976). The uterus of the pig may deliver its luteolytic agent by both local and systemic routes. Unilateral hysterectomy does not affect cycle length in gilts (Ginther and First, 1971). In the gilt,

autotransplantation of the ovary to the body wall or abdominal muscles does not alter length of the estrous cycle (Anderson et al., 1963; Hagen et al., 1981).

Some species do not appear to require uterine influence to control the CL. Removal of the uterus has no effect on luteal function in the rhesus monkey (Burford and Diddle, 1956), human (Beavis et al., 1969) or cynomolgus monkey (Castracane et al., 1979).

The evidence is quite clear that the uterus governs the existence of the CL on the ovary and consequently the estrous cycle (at least in livestock species). But how does it deliver its luteolytic messenger? Both oviductal and neural routes have been suggested and ruled out. Transection of the oviduct in the ewe did not influence life or death of the CL (Baird and Land, 1973). Moore and Nalbandov (1953) demonstrated IUDs failed to induce estrus in ewes when they were placed in denervated sections of the uterine horn ipsilateral to the CL. Observation that ewes continued to cycle normally after separation of the uterus and ovary (Niswender et al., 1970) argues strongly against either pathway.

The only other apparent route would be via the blood supply. McCracken et al. (1971) proposed a "counter-current" mechanism in species with local control of the ovary by the uterus. By this mechanism, there would be a direct venoarterial pathway between a uterine horn and its adjacent ovary. In those species that have a local route of control there is close apposition of the uteroovarian vein (primary vessel of uterine drainage) to the uteroovarian artery. Numerous contacts between vein and artery occur along the tortuous course of the artery over the vein in sheep (Mapletoft and Ginther, 1975) and swine (Oxenreider et al., 1965). There is very little association between these vessels in the mare, a species in which the uterine influence is systemic

(Del Campo and Ginther, 1972; Del Campo and Ginther, 1973). In the cow, as in sheep and swine, the uteroovarian vein and the ovarian artery are very closely associated (Ginther, 1974; Mapletoft et al., 1976). Ginther and Del Campo (1974) reported the uteroovarian arterial anastomosis was significantly more prominent in the side ipsilateral to the CL than on the contralateral side in cattle.

Barrett et al. (1971) infused the luteolytic substance prostaglandin $F2_{\alpha}$ ($PGF2_{\alpha}$) into the ovarian artery of sheep that had ovaries autotransplanted to the neck. This caused CL regression, except in sheep in which the uterine vein was separated from the ovarian artery. Strong support for the hypothesis of transport of the uterine luteolytic agent by the uteroovarian vein is offered by Ginther and Bisgard (1972). Anastomosis between ipsi- and contralateral uteroovarian veins in sheep resulted in CL regression when an IUD was placed in the uterine horn contralateral to the ovary with CL. In similar experiments, Ginther et al. (1973) demonstrated a local uteroovarian venoarterial pathway for uterine induced luteolysis in ewes. In these studies unilateral hysterectomy ipsilateral to the CL bearing ovary resulted in luteal maintenance, but surgical anastomosis of either the main uterine vein or the ovarian branch of the ovarian artery from the intact side to the corresponding vessel on the hysterectomized side resulted in CL regression on the hysterectomized side. When this experiment was conducted with cows the results were the same (Mapletoft et al., 1976).

Interestingly, it has been suggested this local uteroovarian route may also be the mode by which a blood borne luteotrophin from the gravid horn in ewes could reach the ovary and effect its unilateral inhibition of the uterine luteolysin during pregnancy. When the main uterine artery on one side was surgically anastomosed to the corresponding vein on the opposite side (gravid to nongravid in one group and

nongravid to gravid in the other), blood from the gravid side resulted in maintenance of the CL on the nongravid side. Likewise, blood supplied from the nongravid side resulted in luteolysis on the gravid side (Mapletoft et al., 1975).

Coudert et al. (1974a) could find no direct physical connections between uterine venous and ovarian arterial vessels in sheep. In a histological study of the uteroovarian vascular pedicle in sheep no channels between vein and artery could be found (Del Campo and Ginther, 1972). Even though no direct connections have been elucidated, Douglas and Ginther (1973) demonstrated that injection of a relatively small dose (2 mg) of PGF2α locally into the lumen of the uterine horn ipsilateral to the CL in the ewe was more effective than a systemic injection (i.e., there was a local constituent of its transport to the CL). Larger doses worked systemically. This is also true for cows. Only 10 mg PGF2α infused into the uterine lumen of cows resulted in a decrease in plasma progestin concentrations, but a 30 mg injection was required if PGF2α was administered intramuscularly (Chenault et al., 1976).

Hixon and Hansel (1974) reported a selective increase in ovarian artery concentrations (higher amounts than in carotid artery or jugular vein) of the same luteolysin (PGF2α) following intrauterine administration in cows. They attributed this to the preferential transfer of PGF2α from the uteroovarian vein to the ovarian artery. In contrast, other researchers reported when the ovarian artery (in ewes) was sectioned distal to the region where transfer of the uterine luteolytic agent is believed to take place, there was no interruption of the estrous cycle. It was proposed that local transfer could not be the only mechanism by which the luteolysin reached the CL (Lamond and Drost, 1973). In another study, Lamond et al. (1973) reported PGF2α injected into the uterine lumen of cows with sectioned ovarian arteries caused CL

regression. Thus $PGF2\alpha$ was transported by an alternate route (other than local) to the ovary.

Coudert et al. (1974b) could find no transfer of infused ^3H-$PGF2\alpha$ from the uterine vein to the ovarian artery in the ewe. They concluded there was no evidence of active local transport from the uterus to the ovary. McCracken et al. (1972), however, did find that ^3H-$PGF2\alpha$ infusion into the uterine vein, at a point before it joins the uteroovarian vein, was followed by an increase in ^3H-$PGF2\alpha$ in the ovarian arterial blood. More recently, Einer-Jensen and McCracken (1981) found evidence for P4 counter-current transfer in sheep by infusing labelled P4 into the uteroovarian vein close to the hilus of the ovary. Radioactivity levels were higher in the ovarian artery than in the aorta, with an apparent .5% to 1% efficiency of transfer to the ovarian artery. Wolfenson et al. (1985) estimated a 1% transfer efficiency of blood $PGF2\alpha$ from the uterine vein to ovarian artery in cycling cows. Knickerbocker et al. (1986) were also able to demonstrate increased concentrations of $PGF2\alpha$ in the ovarian artery as compared to a peripheral artery in response to estradiol-17ß (E2-17β) in cattle.

$PGF2\alpha$ as the Uterine Luteolysin

From the previously mentioned research, it seems evident the endometrium produces a luteolytic substance that is then conveyed to the ovary by either local or systemic means where it acts on the CL to effect luteolysis. Much of the early work on this substance characterized its actions and predicted its existence, but efforts to obtain luteolytic extracts from uterine contents or venous blood have had variable results.

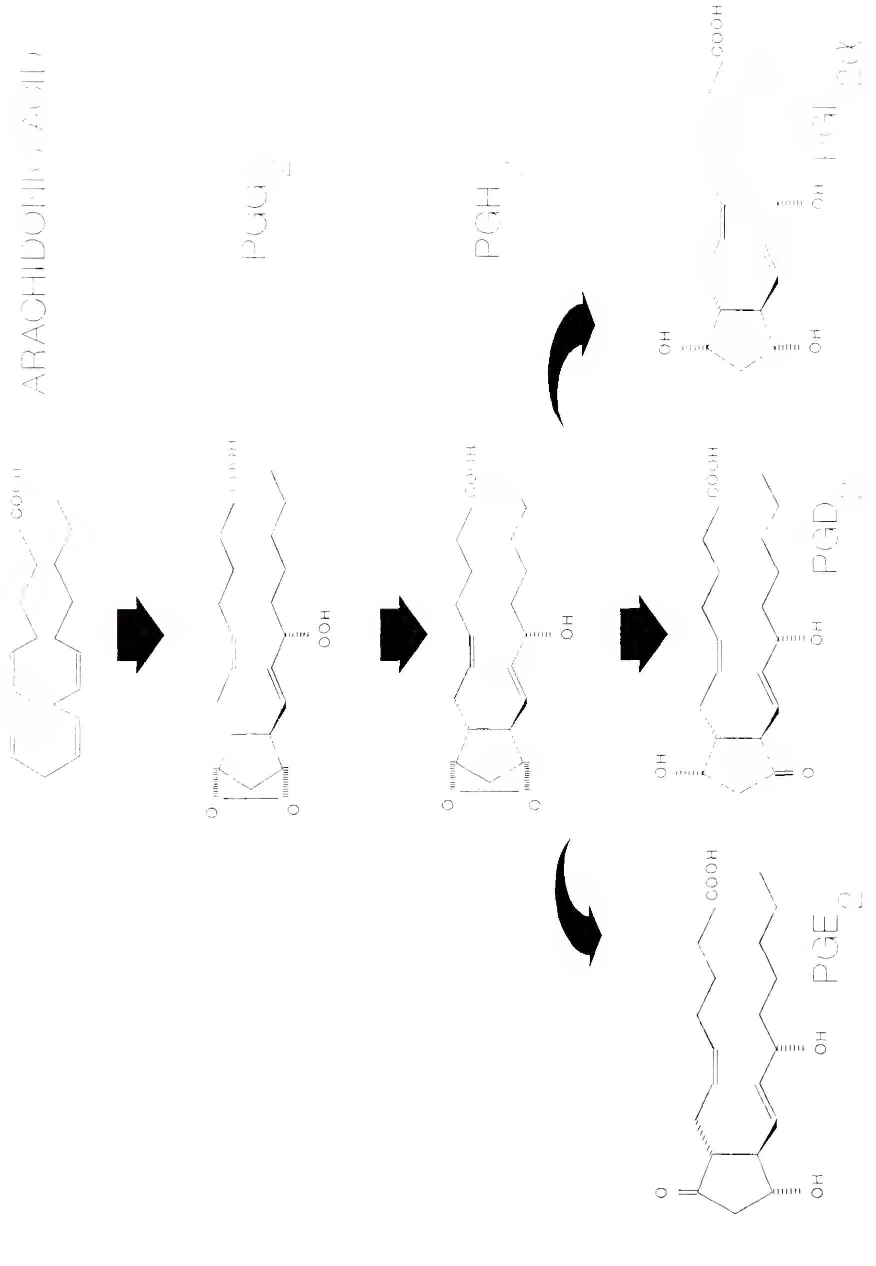
Injections of ether-soluble extracts or lyophilized homogenate of sheep uteri at various stages of the estrous cycle (d 0 or d 4 to 7) failed to promote luteal regression

in hysterectomized ewes (Kiracofe et al., 1966), but aqueous endometrial extracts from the diestrual stage in cows (d 14 and 16) and ewes (d 14 and 15) caused luteal regression in pseudopregnant hysterectomized hamsters (Anderson et al., 1969). Lipid extracts from hamster uteri also caused luteal regression in hamsters (Lukaszewska et al., 1972). Uterine flushings from sows on d 12 to 18 of the estrous cycle caused destruction of pig granulosa cell cultures (i.e., the medium was luteolytic). Flushings from sows on d 1 to 10 or d 20 of the cycle had no effect (Schomberg, 1967). Caldwell and Moor (1971) reported that freeze-dried uterine venous plasma infused into the ovarian artery of ewes precipitated a decrease in ovarian vein P4 and shortened estrous cycle lengths when blood was collected on d 14 but not on d 8.

Babcock (1966) was the first to suggest the luteolytic agent from the uterus might be a prostaglandin. These substances were first isolated from human seminal plasma in the early 1930s (Kurzrok and Lieb, 1930; von Euler, 1934). They were described as producing strong vasodilation and contractions of smooth muscle. Von Euler (1935) gave them the name "prostaglandin" because he erroneously thought that they originated in the prostate gland. They are actually secreted by the seminal vesicle in the male (Setchell, 1977).

Prostaglandins are a family of 20 carbon, monocarboxyllic, unsaturated fatty acids (Walpole, 1975). Position of the oxygen group(s) on the pentane ring determines the series to which a prostaglandin belongs (figure 1). The biologically active prostaglandins are members of the D, E and F series. The number of double bonds in the side chains are indicated by the numerical subscripts 1, 2 or 3. Prostaglandins are found in numerous tissues and mediate a myriad of often contradictory actions (Katz and Katz, 1974).

FIGURE 1. METABOLIC PATHWAY FROM ARACHIDONIC ACID TO THE BIOLOGICALLY ACTIVE PROSTAGLANDINS PGE2, PGD2, AND PGF2α.


ARACHIDONIC ACID
PGG2
PGH2
PGF2α
PGD2
PGE2

The first report of exogenous PGF2α causing CL regression was by Pharriss et al. (1968). Pharriss and Wyngarden (1969) proposed that the uterine luteolytic agent was PGF2α.

Exogenous PGF2α is luteolytic in hysterectomized guinea pigs (Blatchley and Donovan, 1969), rats (Gutknecht et al., 1969; Pharriss and Wyngarden, 1969), hamsters (Lukaszewska et al., 1972), pregnant rabbits (Koering and Kirton, 1973), sheep (McCracken et al., 1970), pigs (Moeljono et al., 1976), horses (Douglas and Ginther, 1972; Allen and Rowson, 1973; Oxender et al., 1974), water buffalo (Kamonpatana et al., 1979), and cattle, either reproductively intact (Lauderdale et al., 1974) or hysterectomized (LaVoie et al., 1975). Species in which the uterus is not necessary for luteal control respond to exogenous PGF2α only under specific conditions. Rhesus monkeys experienced luteolysis when PGF2α was administered for five consecutive days with the first injection on d 11, 12 or 13 of the menstrual cycle (no effect when given on d 7 to 11 or d 4 to 10) (Kirton et al., 1970). In the human, infusion of PGF2α over a 4 h period on d 21 of the cycle resulted in a sharp decline in plasma P4 by 48 h after treatment. By 72 h after infusion plasma P4 concentrations were less than 1 ng/ml and menstruation ensued (Lehmann et al., 1972). Wentz and Jones (1973) observed that PGF2α caused only a transient decline in plasma P4 concentrations when infused for 8 h beginning on d 3, 4, 6, 7, 8, 9, 10, 11 or 12 of the cycle in humans. Plasma P4 concentrations declined by 50% within 12 h after infusion of PGF2α in pregnant women (12 to 14 weeks of gestation) and abortion was induced (Lehmann et al., 1972).

Support for the hypothesis that PGF2α was the luteolysin supplied by the uterus came from observations that distention of guinea pig uteri in vitro (Poyser et al., 1971)

and sheep uteri in vivo (Pexton et al., 1975) resulted in release of PGF2α. Wilks et al. (1972) found PGF2α is synthesized by rabbit uterine tissue in vitro and there is an increase in release rate in tissue obtained from animals during estrus. In the pig, PGF2α concentrations in uterine flushings increase during the luteal phase, reaching peak levels around d 16 of the cycle (Frank et al., 1978; Zavy et al., 1980). Additional evidence for PGF2α as the luteolysin is that ewes and cows passively immunized against PGF2α exhibit prolonged estrous cycles (Fairclough et al., 1981).

Although PGF2α has been shown to be synthesized by the uterus and to be luteolytic when injected in vivo it is not always luteolytic in cultures of luteal cells. Speroff and Ramwell (1970) stimulated P4 production in bovine CL slices incubated with PGF2α and suggested the luteolytic effect of PGF2α when administered in vivo was due to an indirect inhibition of luteal steroidogenesis. PGF2α stimulated P4 secretion and morphological luteinization in rhesus monkey granulosa cell cultures (Channing, 1972) but when rabbit CL tissue was incubated with PGF2α, O'Grady et al. (1972) reported an inhibition of P4 synthesis. Henderson and McNatty (1975) found that small amounts of PGF2α inhibit secretion of P4 by bovine granulosa cells in vitro. They hypothesized that PGF2α initiates functional luteolysis (inhibition of P4 synthesis) by inhibiting synthesis of cyclic adenosine monophosphate (cAMP) in the luteal cell. Whether PGF2α is luteotropic or luteolytic in vitro seems to depend on the milieu of the culture media.

Luteinizing hormone and arachidonic acid are luteotropic when incubated alone in bovine CL cultures (Shemesh and Hansel, 1975b). Prostaglandin F2α increases P4 accumulation in the absence of, but not in the presence of, LH in cultures of bovine luteal cells (Hixon and Hansel, 1979). This is also true for rat luteal cells (Thomas et

al., 1978). Pate and Condon (1984) found no effect of PGF2α on basal concentrations of P4 but reported that PGF2α was able to inhibit LH-stimulated P4 production in vitro cultures of bovine CL. It appears the functional luteolytic effect of PGF2α on cells in culture is upon the agonist-induced P4 production and not the inhibition of basal P4 concentrations.

Theory of Action and Hormonal Regulation of PGF2α

Precisely how PGF2α effects luteolysis is not known. It was first suggested that PGF2α acted indirectly by causing vasoconstriction of vessels to the ovary and the CL subsequently died of ischemia (Pharriss and Wyngarden, 1969). Niswender et al. (1976) found blood flow to the luteal ovary in sheep declined concurrently with P4 concentrations by 6 h after PGF2α treatment. Intravenous administration of PGF2α in ewes was followed by reduced blood flow to the ovary with CL and lower concentrations of systemic P4 (Nett et al., 1976). Other researchers, however, could find no evidence of diminished blood flow despite marked decreases in P4 secretion in response to PGF2α (Behrman et al., 1971; McCracken and Einer-Jensen, 1979). One excellent argument against vasoconstriction as the means of luteolysis is the fact that PGF2α causes functional luteolysis in vitro (Henderson and McNatty, 1975; Hixon and Hansel, 1979).

Alternative theories for the mechanism by which PGF2α precipitates luteolysis involve alteration of receptor number or orientations. Receptors for both LH and PGF2α are found on the plasma membrane of the CL (Powell et al., 1974; Haour and Saxena, 1974; Behrman et al., 1979).

Luteinizing hormone and its receptor form a complex unit that stimulates P4 synthesis by luteal cells. The binding of LH with its receptor causes an increase in

intracellular cAMP concentrations (Henderson and McNatty, 1975). As cAMP concentrations increase, P4 synthesis also increases (Lahav et al., 1976; Behrman et al., 1979; Wakeling and Green, 1981). This increase in P4 synthesis results through the activation of adenylate cyclase, the enzyme that converts adenosine triphosphate (ATP) to cAMP. Cyclic AMP then phosphorylates protein kinases which, in turn, activate the enzymes necessary for P4 synthesis, such as cholesterol esterase (Caffrey et al., 1979). Garverick et al. (1985) reported addition of LH to cultures of bovine luteal tissue (collected on d 7, 10, 13 and 16 of the estrous cycle) increased adenylate cyclase activity relative to basal activity. Activation of adenylate cyclase requires the continued occupation of LH receptors. Dissociation causes deactivation but occupation of only a fraction of the available receptor sites is adequate to cause maximal production of cAMP by the luteal tissue (Koch et al., 1974). Luteinizing hormone increased adenylate cyclase activity in cultures of bovine luteal cells as a result of increased cAMP (Marsh, 1970). Prostaglandin F2α may act to dissociate LH from its receptors, thus causing a decline in cAMP concentrations (Koch et al., 1974) and this, as previously mentioned, may result in the initiation of functional luteolysis (Henderson and McNatty, 1975).

Other ways in which PGF2α could function would be by causing a decrease in LH receptors or blocking their occupation. Hichens et al. (1974) reported PGF2α treatment produced a fall in the binding capacity of rat luteal tissue for human chorionic gonadotropin (hCG) without changing the affinity constants of LH receptors. Incubation with PGF2α did not interfere with binding of LH to membranes containing gonadotropin receptors. They suggested PGF2α may act indirectly via an effect on synthesis, conformation, or breakdown of LH receptors. In sheep, the number of luteal

LH receptors is correlated with luteal weight, P4 content and serum P4 throughout the estrous cycle. The number of LH receptors is lower during early (d 2 to 6) and late (d 16) phases of the cycle than during mid-luteal phase (d 10 to 14). Affinity constant was the same on all days of the cycle (Diekman et al., 1978). Number of luteal LH receptors and P4 concentrations during the estrous cycle are also correlated in cattle (Rao et al., 1979, Spicer et al., 1981). Other reports, however, dispute the concept that luteolysis is preceded by a decrease in LH receptor populations.

In cattle, plasma P4 concentrations are positively correlated with unoccupied LH receptor concentration, basal adenylate cyclase activity, and LH-activated adenylate cyclase activity on d 4, 7, 10, 13, 16 and 19 of the estrous cycle but not with occupied LH receptor concentrations (they remain essentially the same from d 10 through luteal regression). Total occupied LH receptor content, however, is positively correlated with mean plasma P4 concentrations on d 4, 7 and 10, with total occupancy of LH receptors increasing fourfold from d 4 to 10 of the estrous cycle. Total LH receptor occupancy remained unchanged during the rest of the cycle and did not decrease after luteal regression began (Garverick et al., 1985).

The hCG binding capacity of rat CL following injection of PGF2α was not depressed by 2 h after treatment, but serum P4 concentrations were reduced by 70%. The drop in P4 concentrations occurred before the decline in LH receptor number (Grinwich et al., 1976). In ewes, P4 decreased by 7.5 h after PGF2α injection. There were no changes in luteal weight, luteal P4 concentration, total number of LH receptors or number of receptors occupied until 22.5 h post injection. Secretion of P4 by CL decreased well before decreases in occupied or unoccupied LH receptors could be detected (Diekman et al., 1978). This was also the case in cows (Fitz et al., 1980). In

rats, PGF2α caused a decrease in P4 concentrations within 2 h, but LH binding capacity was unchanged. However, removal of the source of gonadotropin releasing hormone (GnRH) by hypophysectomy causes complete and immediate CL regression (Kaltenbach et al., 1968) and reduces LH binding capacity and P4 concentrations within 48 h, indicating the presence of fewer receptors (Behrman et al., 1978).

Alteration in LH receptor numbers may not initiate luteolysis, but it is likely that it is involved in the final destruction of the CL. Progesterone concentrations decrease before any significant morphological changes occur in the CL following treatment of rabbits with PGF2α (Koering and Kirton, 1973). Luteolysis appears to occur in two stages. The first is functional luteolysis in which the CL loses its ability to secrete P4 and the second is structural luteolysis which involves leukocytic infiltration, cellular degeneration and eventual resorption of the CL (Baird and Scaramuzzi, 1975; Behrman et al., 1979). As previously mentioned, decline in LH receptor number and binding affinity comes at some point after decrease in P4 concentrations. Grinwich et al. (1976) proposed the ultimate decline in LH receptors is a mechanism to insure luteolysis continues once started (structural luteolysis). Rao et al. (1984), however, suggested the receptor number decline during bovine luteal regression was an artifact associated with the general deterioration of the cell structural, functional and metabolic integrity.

Luteinizing hormone and its receptors are slow to dissociate once bound (Haour and Saxena, 1974; Henderson and McNatty, 1975), but the effect of PGF2α on P4 secretion by the CL is rapid. Treatment with PGF2α reduced plasma P4 and hCG binding by the CL within 30 min in rats. This led Behrman and Hichens (1976) to suggest PGF2α caused luteolysis by blocking LH uptake. This block on LH binding

could be expected to cause a decrease in adenylate cyclase activity in the luteal cell. There was little effect of PGF2α on adenylate cyclase stimulation in homogenates of bovine CL (Marsh, 1971), but adenylate cyclase activity and cAMP accumulation were inhibited in intact luteal cells (Thomas et al., 1978).

The impact PGF2α has on LH-dependent adenylate cyclase activity is probably not by binding to LH receptors. There are receptors specific for PGF2α on the plasma membrane and there is little cross-reactivity of PGF2α and LH with the non-homologous receptor (Rao, 1975, 1976; Rao et al., 1979).

How then does PGF2α initiate luteolysis? Henderson and McNatty (1975) proposed that PGF2α initiated functional luteolysis by interfering with LH stimulation of cAMP formation. This could result in decreased P4 synthesis. Lahav et al. (1976) reported PGF2α prevented a LH-stimulated rise in cAMP if they were both added to cultures of rat CL at the same time. This also occurred in cultures of human CL (Hamberger et al., 1979). A decrease in luteal adenylate cyclase activity was associated with a decrease in plasma P4 concentrations in sheep (Agudo et al., 1984) and cattle (Fitz et al., 1980) during PGF2α-induced luteolysis.

Other researchers, however, suggest the influence of PGF2α on P4 synthesis occurs at a step after the formation of cAMP. Pate and Condon (1984) reported PGF2α has no effect on basal P4 concentrations in cultures of bovine CL, and P4 synthesis was stimulated by LH or dibutyryl cAMP. The presence of PGF2α in the culture inhibited the LH-stimulated P4 production. This occurred at a site beyond the accumulation of cAMP because dibutyryl cAMP did not increase P4 in the presence of PGF2α. Phosphodiesterase is an enzyme located in the cytosol and membranes of most tissues and is involved in regulation of cAMP concentrations by hydrolyzing cAMP

to 5'AMP (Thompson and Strada, 1978). Garverick et al. (1985) reported an increase in phosphodiesterase activity in bovine luteal tissue on d 19 of the estrous cycle, a time at which adenylate cyclase activity was declining. This relationship between the two enzymes was also reported in sheep within 2 h after PGF2α injection, and the changes in enzyme activity occurred before a decrease in plasma P4 concentrations. It was suggested that a decrease in adenylate cyclase activity (necessary for cAMP synthesis) and an increase in phosphodiesterase activity (responsible for cAMP catabolism) may act in concert to decrease intracellular cAMP concentration and that this decrease in cAMP may be an early event resulting in lowered P4 concentrations during PGF2α-induced (or naturally occurring) luteolysis (Agudo et al., 1984).

Calcium (Ca^{++}) also appears to be involved in the process of luteolysis. There are two groups of PGF2α receptors on the CL, one of which has high affinity binding and the other low affinity binding. Plasma membranes of bovine luteal cells cultured in buffer with no Ca^{++} contain low affinity receptors, but lack high affinity receptors. Addition of Ca^{++} to the media results in the appearance of high affinity receptors. When Ca^{++} is then removed from the culture they disappear. Only high affinity receptors are dependent on Ca^{++}, as low affinity receptor numbers remained constant (Rao, 1975). Rao et al. (1979) suggested CL sensitivity to PGF2α during the cycle is controlled by modulating PGF2α receptor affinity. In bovine CL, there are large numbers of PGF2α receptors present by d 13 of the estrous cycle, but their binding affinity was 203 times lower than at d 20 (about the time CL regression occurs).

Buhr et al. (1979) suggested regression of the CL may involve phase changes in the phospholipid bilayer of cellular membranes. Prostaglandin F2α may be reducing fluidity and increasing permeability of the microsomal and plasma membranes, resulting

in disruption of intracellular enzyme complexes. A gel-phase lipid can be detected in plasma membranes of bovine CL that were removed 24 h after PGF2α injection (Goodsaid-Zalduondo et al., 1982). Corpora lutea from cows in the luteal phase of the estrous cycle had microsomal membranes with all membrane lipids in the liquid-crystalline stage, but samples prepared from regressing CL revealed a phase transition in which some of the lipid bilayer was gel-phase (i.e., less fluidity). Coincident with this physical change was a decline in P4 secretion (Carlson et al., 1982). Carlson et al. (1984) used fluorescence polarization and x-ray diffraction to determine the structural properties of membranes from rat luteal cells. Membrane fluidity was observed to decrease during luteolysis, and this was correlated with a decrease in P4 secretion. This alteration in membrane structure occurs in cells of either spontaneously regressing or PGF2α-regressing CL. Treatment of in vitro cultures of rat CL with PGF2α produces a change similar to that found during spontaneous luteolysis. Polarization increases, which indicates a decrease in membrane fluidity (Riley and Carlson, 1985).

Riley and Carlson (1987) suggest the decreases in fluidity are caused by a synergistic effect of Ca^{++} and hydrolysis products of phospholipase A activity. This decrease in fluidity is probably due to a deterioration of the methylation process. Milvae et al. (1983) suggested methylation of phospholipids within the plasma membranes of luteal cells was an important regulatory step in LH-stimulation of P4 synthesis. They proposed that LH binds to its receptor and stimulates methylation, which in turn, increases membrane fluidity. This increase in fluidity results in the unmasking of more receptors which increases LH binding. An increase in membrane fluidity may increase the probability of the LH-receptor complex interacting with adenylate cyclase.

Phospholipase A2 governs the concentration of arachidonic acid (precursor of PGF2α) in human platelet cells. This process requires the influence of Ca^{++} for maximum activity (Wong and Cheung, 1979). Phospholipase A2 is a water soluble enzyme that catalyzes the hydrolysis of phosphoglycerides to yield a lysophosphotide and an unsaturated fatty acid (typically arachidonic acid) (Riley and Carlson, 1985). Calmodulin and PGF2α stimulate the activity of phospholipase A2 in the presence of Ca^{++} (Moskowitz et al., 1983). In some cells, phospholipid methylation blocks Ca^{++} influx into the cell which results in a decrease in phospholipase A2 activity and arachidonic acid synthesis (Hirata and Axelrod, 1980). Prostaglandin F2α acts to stimulate phospholipase A2, which precipitates an increase in arachidonic acid concentrations. This precursor for PGF2α may enhance the production of the luteolytic substance (via the cyclooxygenase system), which might further accelerate regression in a positive feedback manner (Riley and Carlson, 1985).

Prostaglandin F2α influence on intracellular concentrations of Ca^{++} may also act directly to affect enzyme activity. Calcium will decrease adenylate cyclase activity in luteal cells (Berridge, 1975; Dorflinger, 1978), while it activates phosphodiesterase through calmodulin in brain, heart, lung and testes tissue (Cheung, 1981; Beavo et al., 1982). In rat luteal cell cultures, ovabain (digitalis) and monensin inhibit the acute stimulation of cAMP by LH, probably as a result of influx of Na^{+} into the luteal cell. This increase in intracellular Na^{+} does not directly inhibit adenylate cyclase activity but appears to induce a secondary influx of Ca^{++} which in turn inhibits activation of adenylate cyclase at a site involved in coupling of the receptor to the enzyme. Prostaglandin F2α may act in the same manner as Na^{+}. Maintenance of CL function by LH may result in part by processes that maintain low Ca^{++} levels in the luteal cell

(Gore and Behrman, 1984). Dorflinger et al. (1984) concluded that an acute increase in intracellular Ca^{++} inhibits activation of adenylate cyclase by LH but that this inhibition by PGF2α is not dependent on an influx of extracellular Ca^{++}, but rather is due to an increase in intracellular Ca^{++} by other mechanisms. They suggested intracellular Ca^{++} may increase by the sequestering of Ca^{++} in mitochondria and endoplasmic reticulum or by a decrease in expulsion to the exterior of the cell as well as by an increase in influx from the extracellular medium.

Hormonal Influences and Controls

Substances other than PGF2α have been shown to be luteolytic. Daily injection of estradiol (E2) from d 2 to 12 of the cycle in dairy heifers caused precocious CL regression (Greenstein et al., 1958). Injections of E2 valerate or a natural estrogenic product also caused early CL regression in beef heifers (Wiltbank et al., 1961). The luteolytic properties of E2 are mediated through stimulus of PGF2α release from the uterus. If heifers are hysterectomized (i.e., no PGF2α source), E2 causes a decline in plasma P4 concentrations and P4 content of the CL (Kaltenbach et al., 1964) but does not result in total regression or expression of estrus (Brunner et al., 1969). Estradiol cypionate is an effective luteolytic agent in the intact but not the hysterectomized ewe (Bolt and Hawk, 1975) or heifer (Watson et al., 1980). When estrogen is injected early in the cycle (d 1 to 6) there is no apparent effect on weight or morphology of the CL in the ewe, but when injected on d 9 and 10 of the cycle, CL weight was reduced (Hawk and Bolt, 1970). In the ewe, E2 injection on d 10 of the cycle into the CL caused a decrease in P4 but no change in CL weight (Cook et al., 1974).

During early and mid-cycle the PGF2α concentrations in the bovine endometrium are low (Shemesh and Hansel, 1975a). When PGF2α concentrations are

low or non-existent (due to hysterectomy), E2 influence on CL function is probably through a negative feedback mechanism that decreases the concentrations of circulating luteotropins. However, the decreased concentrations are inadequate to cause total CL regression. During the later phases of the estrous cycle, $PGF2_\alpha$ is present in significant quantity in the uterus and E2 stimulates its release and increased production (Bartol et al., 1981; Knickerbocker et al., 1986). Injections of E2 into cycling heifers caused plasma concentrations of 15-keto-13,14-dihydro $PGF2_\alpha$ (PGFM) to increase with the resultant CL regression (Thatcher et al., 1986). Plasma $PGF2_\alpha$ is inactivated during passage through the pulmonary circulation (probably by 15-hydroxyprostaglandin dehydrogenase) and forms the metabolite, PGFM (Piper et al., 1970). If indomethacin, a substance that inhibits $PGF2_\alpha$ synthesis by the endometrium (Lewis and Warren, 1977), is injected in heifers along with E2 benzoate, it prevents the expected induced CL regression. This would suggest the luteolytic action of estrogen is by increased $PGF2_\alpha$ synthesis and its release from the uterus (Warren et al., 1979).

Some researchers have demonstrated that the E2 stimulated synthesis and release of $PGF2_\alpha$ from the endometrium must be preceded by P4 priming of the uterus (Caldwell et al., 1972; Barcikowski et al., 1974; Scaramuzzi et al., 1977). Spontaneous E2 peaks occur throughout the cycle, but it is not until the time of CL regression that peaks of $PGF2_\alpha$ are correlated with E2 peaks. $PGF2_\alpha$ is released only in late luteal phase from an autotransplanted uterus following injection of E2. This would indicate P4 priming is necessary to $PGF2_\alpha$ synthesis (Roberts et al., 1975).

Estrogen appears to have a role in spontaneous CL regression. Destruction of all visible follicles (the source of endogenous estrogen) on both ovaries in ewes resulted in delayed CL regression following IUD insertion into the uterus of ewes.

Cauterization of the follicles on only one ovary did not result in delayed regression (Ginther, 1971). Cook et al. (1974) reported injected E2 caused CL regression in ewes when ovaries had all follicles destroyed, but only if injected into the ipsilateral ovary and not the contralateral one. Progesterone concentrations were maintained past the expected time of luteolysis in ewes (Hixon et al., 1975) and in cows that had all follicles on the ovaries destroyed at some time during mid-cycle (Fogwell et al., 1985). It was suggested that E2 initiated luteal regression, possibly by involvement in $PGF2_{\alpha}$ release.

The other factor involved in luteolysis is oxytocin (OT). Armstrong and Hansel (1959) found that administration of OT by subcutaneous or intravenous injections daily from d 0 to 7 of the estrous cycle in dairy heifers shortened cycle length to 8 to 12 d. They concluded OT caused inhibition of CL function possibly by interfering with the secretion of a luteotrophic hormone from the pituitary. However, as in the case of estrogen, the luteolytic effect of OT is probably mediated through $PGF2_{\alpha}$ release from the uterus. Administration of exogenous OT shortened estrous cycle length if heifers were reproductively intact or if the contralateral uterine horn was removed. Removal of the ipsilateral horn prolonged the estrous cycle (Ginther et al., 1967).

Like estrogen, OT injection early (d 0 to 4) or late (d 15 to 22) in the estrous cycle has no effect on cycle length, but injection during the luteal phase results in CL regression (Hansel and Wagner, 1960; Black and Duby, 1965). It is likely that injections of OT administered earlier than d 5 of the cycle, as in a study by Armstrong and Hansel (1959), are superfluous. A later study (Hansel and Wagner, 1960) demonstrated that injections given on d 0 to 2 or d 0 to 4, inclusive, failed to shorten the estrous cycle in dairy heifers. Administration of OT injections on d 3 to 6 was as effective as injections given from d 0 to 7.

When physiological amounts of OT are infused into the arterial supply of the uterus in ewes, the tone of the uterus and amplitude of contractions increase and are associated with a simultaneous release of PGF2α (Roberts et al., 1975). A single injection of OT resulted in an increase in plasma PGFM when given to ewes on d 14 of the estrous cycle but not on d 3 or 8 (Fairclough et al., 1984). Lafrance and Goff (1985) reported a single injection of OT on d 17, 18 or 19 of the cycle in heifers precipitated an increase in PGFM but had no effect when the injections were given on d 6 or 13. In contrast, multiple injections of OT did elicit PGF2α release earlier in the cycle. Treatment of heifers with OT on three consecutive days beginning on d 3 of the estrous cycle resulted in increased concentrations of PGF2α in the peripheral blood supply (Newcomb et al., 1977). Daily OT injections on d 4, 5 and 6 or d 5 and 6 caused shortened estrous cycles and increased uterine venous PGF2α concentrations in heifers (Milvae and Hansel, 1980). Injections given twice daily from d 2 through 6 of the estrous cycle resulted in increased plasma PGFM concentrations on d 2 and 3 and a shortened cycle in two of six cows treated. All cows treated in this manner had a slower P4 increase through d 8 of the estrous cycle than controls (Oyedipe et al., 1984). Administration of OT on d 3 to 6 of the cycle in goats also results in elevated plasma PGFM concentrations with accompanying P4 decline (Cooke and Homeida, 1982).

Flint and Sheldrick (1985) reported continuous infusion of OT between d 13 and 21 of the estrous cycle in ewes delayed return to estrus by 7 d. Progesterone also remained high, indicating luteal regression was prevented. Continual infusion of OT during this phase of the cycle prevented the rise in uterine OT receptors which normally precedes estrus, possibly by down-regulation. This may result in an inhibition

of PGF2α synthesis or release from the endometrium. However, continuous infusion of cattle with OT from d 14 to 22, d 15 to 18, or d 16 to 19 did not significantly affect luteolytic events (Kotwica et al., 1988).

Oxytocin enhanced PGF2α release from cultures of endometrium. The number of high affinity OT receptor sites on the endometrium and myometrium were at their peak in cultures of these tissues from ewes at estrus (Roberts et al., 1976). Mean OT receptor concentrations in caruncular and intercaruncular endometrium and myometrium increased from d 10 to estrus in cycling ewes. This increase in receptors coincided with luteolysis and the concomitant decrease in P4 (Sheldrick and Flint, 1985). As in sheep, endometrial OT receptor concentrations in heifers are low during the luteal phase of the estrous cycle, but increase rapidly during luteolysis and reach a maximum at estrus (Meyer et al., 1988). Myometrial plasma membranes bound nearly ten times more OT when the tissue was collected on d 21 of the cycle than when collected on d 7 (Soloff and Fields, 1989).

Endogenous OT concentrations increase following PGF2α injection in ewes (Flint and Sheldrick, 1983) and cows (Schams and Karg, 1982; Schallenberger et al., 1984). When production of endogenous PGF2α in vitro was suppressed with indomethacin, the myometrium responded normally to OT, demonstrating that increased synthesis of PGF2α is not essential for activation of the myometrium by OT (Roberts and McCracken, 1976). Tritschler et al. (1983) found OT promoted luteolysis in all cows treated and this effect was not blocked by indomethacin, suggesting that increases in uterine PGF2α synthesis may not be responsible for OT-induced luteolysis, but that OT may act to initiate release of PGF2α. In contrast, Cooke and Knifton (1981) reported subcutaneous injections of OT caused induction of estrus in goats, but administration

of meclofenamic acid (a prostaglandin synthetase inhibitor) inhibited this luteolytic effect. Active immunization of ewes against OT prolonged the luteal phase of the estrous cycle (Sheldrick et al., 1980; Schams et al., 1983).

Oxytocin is a nonapeptide hormone generally thought of as being produced by the hypothalamus and released from the posterior pituitary (Wathes and Swann, 1982). Early in this century, Ott and Scott (1910) reported the corpora lutea of goats contained an oxytocic-like substance. More recently, extracts of ovine (Wathes and Swann, 1982; Theodosis et al., 1986) and bovine (Fields et al., 1983; Wathes et al., 1983) CL have been shown to contain OT. Large quantities of mRNA for OT exist in the bovine CL during mid-luteal phase of the estrous cycle. This mRNA for luteal OT is very similar to mRNA for hypothalamic OT, but an active CL produces approximately 250 times more OT mRNA than a single hypothalamus (Ivell and Richter, 1984). The CL is the primary site of ovarian OT (Flint and Sheldrick, 1982), but Ivell et al. (1985) reported finding mRNA for OT detectable at low concentrations in mid-cycle follicles. Other researchers reported the measurement of immunoreactive OT in the follicles of cycling cattle (Wathes et al., 1984; Kruip et al., 1985; Schams et al., 1985; Wise et al., 1986).

Corpora lutea from ewes (Fitz et al., 1982) and cows (Priedkalns and Weber, 1968; Koos and Hansel, 1981; Weber et al., 1987) contain two populations of luteal cells. One population consists of large cells (> 23 μm in diameter) and the other of small cells (12 to 23 μm in diameter). Interestingly, immunoreactive OT or OT-associated neurophysin is contained in large luteal cells and not small cells of cycling ewes (Rodgers et al., 1983; Fields and Fields, 1986) and cows (Guldenaar et al., 1984; Fields and Fields, 1986). Only the large cell of bovine CL contains mRNA for OT (Fehr

et al., 1987). Immunoreactive OT and OT-associated neurophysin could not be found in the large luteal cells of pregnant cows (Guldenaar et al., 1984).

These same large cells also contain the majority of receptors for PGF2α (and coincidently, PGE2) and the fewest receptors for LH/hCG when compared to small luteal cells in cycling ewes (Fitz et al., 1982). Large cells contain and secrete most of the P4 produced by the CL, but small cells demonstrate an increase in P4 synthesis and secretion in response to LH challenge in cultures of luteal tissue from the mid-cycle cow (Ursely and Leymarie, 1979; Koos and Hansel, 1981) and ewe (Fitz et al., 1982). Harrison et al. (1987) reported the basal P4 production by large cells of mid-cycle ovine CL was 6 to 8 times higher than that of small cells. Addition of LH to separate cultures of these cells stimulated P4 production by small cells, but not large cells. However, when small and large cells were recombined in a single culture the effect of addition of LH was synergized and the combined culture produced more P4 than cultures of the small cells alone.

Gemmel et al. (1974) reported granules were present in the cytoplasm of luteal cells of the ewe and their numbers increased as the estrous cycle progressed. This is correlated with the rise and decline of P4 during the cycle (Heath et al., 1983). The peptide hormones neurophysin and OT have been demonstrated to be present in electron dense granules within the large luteal cell (Fields and Fields, 1986; Theodosis et al., 1986; Fields et al., 1989). It has been theorized that these or other electron dense granules may also contain sequestered P4 and that this is probably the method of P4 release from the large luteal cells (Gemmel and Stacy, 1979; Quirk et al., 1979). Rice et al. (1986) demonstrated approximately 30% of total P4 in ovine CL is associated with subcellular granules, but that the particle associated P4 does not have

similar physical or biochemical characteristics to OT containing granules. Luteal granules that do contain OT displayed physical and biochemical characteristics similar to those reported for neurohypophysial OT granules except that luteal granules were 1.3 times larger in diameter (Rice, 1988). Injection of PGF2α in sheep (Stacy et al., 1976) or cattle (Heath et al., 1983; Braun et al., 1988) resulted in decreases in the relative percentages of cytoplasm occupied by granules in large luteal cells, but not small luteal cells. Similar observations were made when bovine luteal slices were incubated with PGF2α (Chegini and Rao, 1987).

Wathes and Swann (1982) hypothesized the OT in the peripheral plasma could be of luteal origin because its increase and decrease correspond to growth and regression of the CL. Flint and Sheldrick (1982) demonstrated that injections of PGF2α in sheep produced a secretion of OT into the uteroovarian vein. Pulses of OT, neurophysin and PGF2α were measured in blood samples collected at hourly intervals from the uteroovarian vein draining the CL in sheep on d 13 to 16 of the estrous cycle (Hooper et al., 1986). In addition, plasma OT concentrations decrease with ovariectomy and episodic release is not detected during seasonal anestrus in sheep (Sheldrick and Flint, 1981; Schams et al., 1982).

Oxytocin and estrogen are closely aligned in their luteolytic effect on the CL. In ovariectomized ewes, OT alone could not effect PGF2α release from the uterus. Injection of E2 alone increased PGF2α concentrations 3 fold, but when OT was injected into E2 primed ewes, PGF2α concentrations rose 30 fold (Sharma and Fitzpatrick, 1974). As previously mentioned, P4 priming also appears to be necessary for the synthesis and release of PGF2α from the endometrium (Roberts et al;, 1975). Oxytocin injections caused increases in plasma PGFM in ovariectomized heifers after 7, 14 or 21

d of P4 priming. The OT induced PGFM increase after 14 or 21 d of P4 priming was higher at 6 h after E2 injection than before the injection. It was suggested that under the influence of P4, E2 enhances the OT-induced release of PGF2α and that there was a possible synergistic action of these hormones in the induction of luteolysis in heifers (Lafrance and Goff, 1988).

This synergism between estrogen and OT may be mediated through estrogen and(or) OT receptor regulation. Increases in estrogen produce increases in OT receptors on the endometrium. As these receptors become occupied with OT, which is present at basal levels in the peripheral circulation, they induce PGF2α release from the uterus, which may result in initiation of luteolysis. Prostaglandin F2α causes the release of OT from the CL and this OT may reinforce the further secretion of PGF2α from the uterus. Receptors for OT may be down-regulated by the release of OT. As the receptors for OT are regenerated they may cause the further episodic releases of PGF2α from the endometrium (McCracken et al., 1984). Luteolysis is accompanied by a decline in P4 concentrations, which would result in decrease of the negative feedback control of P4 on estrogen receptors. Increasing occupation of the estrogen receptors would elicit increased numbers of OT receptors and the resultant occupation of those receptors with subsequent PGF2α release could cause the final luteolysis of the CL (Leavitt et al., 1985).

Other researchers suggest that measurements of plasma OT at about the time of luteal regression do not support the theory of increased release of PGF2α in response to peripheral OT. Webb et al. (1981) reported plasma OT concentrations in the ewe (in blood samples collected every 3 h) decreased around the time of CL regression, preovulatory gonadotropin surge and beginning of the next luteal phase.

This was in contrast to increased concentrations of PGFM occurring during luteal regression. Sheldrick and Flint (1981) reported an increase in basal concentrations of OT in the ewe (in blood samples collected once a day), but they suggested it was unlikely to cause the rapid increase in uterine release of PGF2α at the end of the estrous cycle. Oxytocin concentrations in bovine ovaries increased from d 1 to 10 of the cycle and then declined from d 11 to 20, before a decline in P4 occurred (Wathes et al., 1984).

But release of OT from the CL occurs in a pulsatile fashion and is associated with the release of PGF2α from the endometrium in the ewe (Flint and Sheldrick, 1983) and cow (Schams et al., 1985). Fairclough et al. (1983) reported coincident surges of OT-associated neurophysin and PGFM in plasma during luteal regression in ewes. However, in a subsequent study, injection of OT in ewes on d 14 of the cycle produced a rise in PGFM concentrations but no consistent increase in OT-associated neurophysin (Fairclough et al., 1984). They concluded that because only 1 of 4 ewes had a significant rise in OT-associated neurophysin following OT injection the data did not support the view that endometrial release of PGF2α stimulated OT release from the CL. Conversely, daily injections of indomethacin (a prostaglandin synthetase inhibitor) on d 11 to 16 of the estrous cycle in goats suppressed the decline in basal concentrations of OT and the pulsatile appearance of OT and PGFM in peripheral circulation. This would suggest PGF2α may stimulate the pulsatile release of OT at luteolysis (Cooke and Homeida, 1984). Abdelgadir et al. (1987) demonstrated that PGF2α did induce OT release by bovine CL in vitro if the CL was collected on d 8, but not d 12 to 16, of the estrous cycle. The addition of PGF2α to cultures of ovine CL, however, had no effect on secretion of OT (Hirst et al., 1986, 1988). Hooper et al.

(1986) found most PGF2α pulses measured in plasma samples collected at hourly intervals from d 13 to 16 in cycling ewes coincided with pulses of OT. Hixon and Flint (1987) reported the administration of E2-17β on d 9 and 10 of the estrous cycle in ewes raised OT receptor concentrations in caruncular endometrium and myometrium by 12 h, followed by an increase in peripheral plasma OT by 26 $\pm$ 3 h, an increase in plasma PGF2α by 35 $\pm$ 3 h, and a decrease in plasma P4 by 42 $\pm$ 3 h.

Concentrations of PGF2α in the uteroovarian vein of ewes during luteolysis began to increase before concentrations of OT and OT-associated neurophysin increased (by an average of 17 min) (Moore et al., 1986). This supports the theory that endometrial PGF2α initiates the release of ovarian OT during luteolysis. If this is the case, OT may provide positive feedback on PGF2α release and cause down-regulation of uterine OT receptors to fine tune PGF2α pulses so they can cause CL regression more efficiently (Schramm et al., 1983). Luteal OT probably reaches the endometrium in the same local transfer manner that results in transport of endometrial PGF2α to the CL. Radioactively labelled OT (^{125}I-OT) was exchanged locally from the uteroovarian vein to the ovarian artery in sheep with a transfer rate of approximately 1% (Schramm et al., 1986). Currently, evidence from the above data is unable to prove conclusively that ovarian OT precipitates luteolysis by initiating PGF2α release from the endometrium or, alternately, that PGF2α initiates the release of ovarian OT to achieve luteal regression.

Some researchers have suggested OT in the CL may be involved in limiting luteal P4 secretion by a local mechanism (Flint and Sheldrick, 1982; Wathes et al., 1983). Cultures of bovine luteal cells responded to low levels of OT with an slight enhancement of P4 production. Higher concentrations of OT, however, resulted in an

inhibition of basal and hCG stimulated P4 production (Tan et al., 1982). Flint et al. (1989), however, suggest it is possible that impurities in OT prepartions are responsible for the stimulatory and inhibitory effects reported for in vitro cultures. They suggest evidence supporting a systemic role of oxytocin in the control of luteolysis is the fact that concentrations of oxytocin receptors in caruncular and inter-caruncular endometrium rise as plasma P4 concentrations fall during luteolysis in ewes (Sheldrick and Flint, 1985) and that either OT receptor concentrations rise as a result of the declining P4 concentrations or the rise in OT receptor concentrations is a cause of luteal regression (Flint et al., 1989).

In addition to the peptide hormone OT, the CL of sheep have been shown to contain PGF2α (Patek and Watson, 1974; Rexroad and Guthrie, 1979). Shemesh and Hansel (1975c) reported in vivo injection of arachidonic acid into the bovine CL produced a decline in P4 and increase in PGF2α and estrogen concentrations in the ovarian vein draining the CL, indicating the synthesis of PGF2α by either the ovarian or luteal tissue. In culture, the bovine ovary synthesizes PGF2α in both follicular and luteal tissue (Shemesh and Hansel, 1975b). It has been suggested that local production of PGF2α by the CL may result in its ultimate regression (Patek and Watson, 1976; Rothchild, 1981). Chronic intraluteal administration of PGF2α caused luteolysis in rhesus monkeys, leading the authors to suggest the data supports the hypothesis that local production of PGF2α initiates normal CL regression (Auletta et al., 1984).

As previously discussed, exogenous PGF2α is luteolytic in domestic livestock species. Regardless of the exact mechanism by which PGF2α induces luteolysis,

practical application of its effect has been used to synchronize estrus in cattle production enterprises.

Practical Use of $PGF2\alpha$ for Estrus Synchronization

Soon after the first report that exogenous $PGF2\alpha$ was luteolytic in pseudopregnant rats (Pharriss and Wyngarden, 1969) researchers began exploring its potential use for control of the estrous cycle in cows. Administration of $PGF2\alpha$ by subcutaneous or intramuscular injection (Lauderdale et al., 1974) or by infusion into the uterus (Louis et al., 1974) of the cycling cow or heifer resulted in premature expression of estrus in most of the treated animals. Similar results were obtained when synthetically produced analogs were used (Tervit et al., 1973; Cooper, 1974). Cycling animals treated with $PGF2\alpha$ generally expressed an induced estrus by 68 to 80 h after administration of the drug (Louis et al., 1974; Henricks et al., 1974; Chenault et al., 1976; Stellflug et al., 1977; Renegar et al., 1978). Some researchers, however, reported shorter average intervals to estrus of 40 to 62 h (Galina et al., 1982; Gonzalez et al., 1985; Graves et al., 1985). Part of this difference may be due to the subjective determination of time of estrus, but interval to estrus is also influenced by stage of the estrous cycle at which $PGF2\alpha$ is injected. Cows and heifers injected at an early point in their estrous cycle have shorter intervals to estrus than those injected late in the cycle (Macmillan, 1978, 1983; Tanabe and Hann, 1984; Watts and Fuquay, 1985).

Fertility to artificial insemination following a single injection of $PGF2\alpha$ did not differ or was slightly higher than controls (Roche, 1974; Day, 1977; Gonzalez et al., 1985, Wahome et al., 1985) when animals were AI according to the AM/PM rule first proposed by Trimberger (1948). This rule requires that all animals expressing estrus in the morning (AM) be AI in the evening (PM, approximately 12 h later) and, likewise, all

animals exhibiting estrus in the PM be AI during the following AM. Use of this system is labor intensive as it makes imperative a careful visual appraisal of the treated animals at least twice daily during the anticipated breeding period.

An alternative would be the AI of all treated animals at an appointed time after injection of PGF2α. One obstacle to this system was evident from the first studies using PGF2α in cattle. Cows or heifers that were on d 0 (estrus) to 5 of the estrous cycle failed to demonstrate response to an injection of PGF2α by exhibiting a premature estrus (Inskeep, 1973; Henricks et al., 1974; Ellicott et al., 1975). Therefore, in a group of randomly cycling cows, approximately 25% (at any one time) will be at a point in their cycle when an injection of PGF2α is ineffective. One method for circumventing this problem was to inject only those cows that were known to be on d 6 or later of the cycle (as determined from date of previous estrus). Another was to treat only those animals with a CL of adequate size to be rectally palpable (Lauderdale et al., 1974) or to produce concentrations of P4 indicative of diestrus (Turman et al., 1975). When animals were treated selectively, some researchers reported no difference in pregnancy rates between cows AI according to estrus or those AI at pre-set times after injection (Lauderdale et al., 1974; Plunkett et al., 1984). Others indicated a tendency for lower pregnancy rates to timed AI when compared to AI by the AM/PM rule (Turman et al., 1975; Hardin et al., 1980b).

Even when AI by appointment was successful, this system required a great deal of time and effort to assure animals were at the proper phase of their estrous cycle to achieve response to an injection of PGF2α. Roche (1974) proposed a system using two injections of PGF2α given with a 10 to 12 d interval between injections. Theoretically, this system would make sure that all cycling animals treated would be at

the proper stage of the estrous cycle to respond to a second injection. As previously mentioned, randomly cycling cows at d 0 to 5 would not respond to the first injection, but cows on d 6 to 21 could be expected to express estrus (either natural or induced) within 3 to 4 d after initial treatment. Ten to 12 d later, at the time of the second injection, cows which had not responded to the first injection would be at d 10 to 17 of the cycle (a phase during which they should respond) and cows that had expressed estrus after the first injection would be at approximately d 6 to 9, again at a stage of the cycle when they should be responsive to PGF2α. Some researchers have reported outstanding success with this system. In these cases over 90% of treated animals (cattle that had functional CL prior to treatment) expressed a synchronized estrus after the second injection (Cooper, 1974; Dobson et al., 1975; Leaver et al., 1975; Adeyemo et al., 1979; Jöchle et al., 1982; Kiracofe et al., 1985; Adeyemo, 1987).

Other studies produced response rates that were lower than should be expected when treating only cycling animals. These studies reported that 11 to 36% of those treated failed to express estrus after the second injection (King and Robertson, 1974; Britt et al., 1978; Burfening et al., 1978; Ansotegui et al., 1983). Field trials of the two injection protocol also yielded lower response rates of 52 to 73% (Lauderdale et al., 1981). A somewhat lower response rate would not be unexpected as field trials involve treatment of entire herds which would contain both cycling and non-cycling cows. However, lack of response by non-cycling animals may not entirely account for a low response rate after the second injection. When cows were treated using the two injection system with a 12 d interval 62% responded to the second injection. Of the cows that were treated, 15% were found to be non-cycling and 23% were cycling but failed to be synchronized (Hafs and Manns, 1975). Donaldson et al. (1982) reported 93

of 237 treated cows showed estrus after the first injection, but 37.6% of those responding to the first injection failed to respond to the second. It can legitimately be argued that estrus detection is a subjective system for measuring response rate (and therefore subject to errors of interpretation) or that cows may experience luteal regression without expression of estrus, but when P4 concentrations were used as an indicator of CL function in dairy cows, 23 of 176 (13%) with high concentrations of P4 failed to experience luteal regression after the second injection (Stevenson et al., 1987). In other words, these animals "should" have responded, but did not (Lucy et al., 1986).

The apparent difficulty in synchronization with the two injection system is not limited to cows. Smith et al. (1984) used injections of PGF2α to synchronize Holstein heifers, all of which were cycling prior to treatment. They reported a significant number (16%) of treated animals were not observed in estrus after the second injection. When these non-estrual heifers were AI at 80 h post-injection only one conceived. Overall pregnancy rate of the PGF2α treated and timed AI heifers (52%) was lower than in controls bred at a naturally occurring estrus (73%). Differences in the pregnancy rates were attributed to 1) poor synchrony of estrus, 2) failure of a significant number of heifers to respond to the second injection and(or) 3) improperly timed inseminations rather than to reduced fertility in the treated heifers.

As in the case of breeding by appointment after a single injection of PGF2α, pregnancy rates to timed AI following use of the two injection system varied greatly. Some researchers reported no difference in rates between animals bred by the AM/PM rule and those AI at set times (Hafs et al., 1975; Manns et al., 1976; Waters and Ball, 1978; Roche and Prendiville, 1979; Kazmer et al., 1981; Jöchle et al., 1982). Conversely, others reported timed AI after synchronization with two injections of PGF2α

(with an interval of 10 to 12 d between injections) resulted in lower pregnancy rates than AI according to estrus (Ellicott et al., 1975; Roche, 1976; Moody and Lauderdale, 1977; Donaldson, 1977; Hardin et al., 1980a; Graves et al., 1985; Stevenson et al., 1987). Short et al. (1978) concluded inseminating at predetermined times following synchronization lowered pregnancy rates, but when breeding was done in relation to estrus, pregnancy rates after PGF2α are similar to unsynchronized AI and natural service. Other studies reported no difference in pregnancy rates to AI to estrus after synchronization using the two injection system and AI to a natural estrus if insemination was performed according to estrus expression (King and Robertson, 1974; Lauderdale et al., 1980; Hardin et al., 1980a; Lauderdale et al., 1981; Neuendorff et al., 1984; Kiracofe et al., 1985). Macmillan and Day (1982) and Macmillan (1983) went so far as to suggest PGF2α enhanced fertility if AI was performed according to estrus. Dairy cows treated with two injections of PGF2α at an 11 d interval had pregnancy rates of 69% compared to 60% in untreated herdmates.

As suggested by Smith et al. (1984), unsatisfactory results to timed insemination may result from a lack of synchrony after treatment. Johnson (1978) and Refsal and Seguin (1980) reported synchrony of estrus was more precise after the second injection than after the first in a two injection scheme. The interval to estrus after a second injection is also shorter (Johnson, 1978; Burfening et al., 1978; Hardin et al., 1980b). Although this is what is desired in a timed insemination program, the increase in degree of synchrony may not be adequate to assure successful timed AI. Interval to estrus is shorter in cattle injected in early diestrus than in those injected during late diestrus (by approximately 12 h) (King et al., 1982; Stevenson et al., 1984). Jackson et al. (1979) reported cows injected on d 7 to 8 or d 15 to 16 had shorter intervals from

injection to LH peak and estrus than animals injected on d 12 to 14. This effect of stage of cycle at time of treatment on interval to estrus could result in fewer animals in a herd being at the correct stage of estrus for timed AI. Donaldson et al. (1982) reported that only 55.7% of the cows that responded to injections of PGF2α expressed estrus in the time frame necessary for AI by appointment.

Synchronization and AI in the Brahman

The Brahman was developed in the U.S. just after the turn of the century by crossing four breeds of Bos indicus cattle. The four breeds, Kankrej (Guzerat), Krishna Valley, Ongole (Nellore), and Gir, were imported from India and Brazil largely between the years 1900 and 1946 (Brockett, 1977). The American Brahman Breeders Association (ABBA) was established in 1924. Its first secretary, J. W. Sartwelle, proposed the name Brahman for the breed (Saunders, 1980).

The Brahman and other Zebu breeds are used in purebred and crossbreeding programs in the tropics and sub-tropics because of their ability to adapt to hot, humid climates and to flourish under conditions of insect infestations and enzoodic diseases that prove fatal to many Bos taurus breeds (Fowler, 1969). The southern states were once considered the poorest beef producing region in the U.S. because of this type of environment. Expansion of improved pastures and use of Brahmans in crossbreeding programs have been credited with the extensive increase in beef production in this section of the country (Fowler, 1969). Frequently, AI is the method by which crossbreeding has been accomplished.

Naturally bred Brahmans have been reported to have lower pregnancy rates when compared to Bos taurus breeds (Burns et al., 1959; Kincaid, 1962; Koger et al., 1973; Crockett et al., 1978). This has also been reported in Brahmans that have been

AI after PGF2α synchronization. Tucker et al. (1982) found the pregnancy rate to AI at estrus following PGF2α synchronization was lower in purebred Brahmans than in commercial Angus, Hereford or Simmental cows (20.8% vs 61.5%, 66.6% and 61.5%, respectively). Zebu cows treated with PGF2α had lower pregnancy rates to AI than untreated controls, but treated cows were AI by appointment at 80 h after injection and controls were AI according to the AM/PM rule (Landivar et al., 1985). The poor pregnancy rate after PGF2α may have resulted from improper timing of AI as the responding treated cows exhibited estrus at 46 to 54 h after injection. Gilson et al. (1981) reported a higher pregnancy rate to AI at 8 to 16 h after induced estrus than to timed AI at 80 h in high percentage Brahman crossbred cows. The average interval to estrus following a single injection of alfaprostol (a PGF2α analog) during the luteal phase of the cycle (approximately d 12) in Brahman heifers and cows was 89 h. A tight synchrony of estrus did not result and only 13% of the treated animals would have been in the correct time frame for optimum fertility to timed AI (Hansen et al., 1987a).

Low pregnancy rates to timed AI after PGF2α synchronization may result from a poor response (as measured by rate of estrus) after treatment. In Zebu cows, only 59% of animals with a palpable CL prior to a single injection of PGF2α expressed estrus following treatment (Orihuela et al., 1983). Other studies have indicated a poor response rate in Zebu or Zebu crossbred animals (Galina et al., 1982; Landivar et al., 1985). Use of the two injection system of PGF2α synchronization has also resulted in inadequate response rates. Purebred cycling Brahmans expressed estrus 46.0% and 46.4% of the time after the first and second injection, respectively (Neuendorff et al., 1984). Nagaratnam et al. (1983) reported response rates of 47% and 76% following

the first and second injections in cycling White Fulani and Sokoto Gudali cattle. Estrus expression may not be the best indication of actual response to PGF2α treatment. Moreno et al. (1986) observed estrus in only 47% of treated Zebu cattle in one experiment and 60% of those in a second experiment, but palpation of the ovaries and plasma P4 at 70 h after injection indicated most animals had experienced CL regression. They concluded that PGF2α was luteolytic in Zebu cattle although estrus expression after treatment was poor. Some researchers have reported excellent response rates in Zebu cattle to PGF2α treatment (Adeyemo et al., 1979; Gilson et al., 1981).

Still, as previously mentioned, pregnancy rates to either timed AI or AI to estrus have been reported to be low in Brahman or other Zebu animals. Brahmans have been reported to have smaller CL, less P4/CL, and lower plasma P4 concentrations on d 2 to 11 of the estrous cycle than Herefords (Irvin et al., 1978; Randel, 1984). Brahman cows also have lower serum P4 concentrations on d 7 to 17 of the cycle than Angus cows (Segerson et al., 1984). When Brahman cows were injected with cloprostenol (a PGF2α analog) the CL that resulted after the induced estrus was smaller and contained less P4 than the CL formed after a natural estrus (Hardin and Randel, 1982). A single luteolytic dose of cloprostenol administered at mid-cycle (d 8 to 12) reduced the weight and total P4 content of the subsequently developing CL in Brahman cows when compared to the CL after a natural estrus. Plasma concentrations of P4 on d 2 to 13 after a cloprostenol induced estrus were lower than in controls (Hardin and Randel, 1982).

Hansen et al. (1987b) also reported the formation of a subfunctional CL in Brahman heifers and cows following treatment with another PGF2α analog, alfaprostol.

Brahman females were given a single injection of alfaprostol on d 12 ± 0.2 of a spontaneous estrous cycle and the corpora lutea were removed on d 13 of the induced estrous cycle. All of the females treated with a dose of 2.25 mg/100 kg bodyweight had lower serum P4 concentrations on d 3, 4, 10, 11, and 12 of the induced estrous cycle when compared with controls. Corpora lutea formed following treatment with alfaprostol produced lower in vitro P4 concentrations in response to LH than corpora lutea formed after a spontaneous estrus. It was suggested the low fertility in Brahman or Brahman crossbred cows could be caused by impaired CL development or other direct ovarian effects.

Effect of Plasma Progesterone Concentrations on Pregnancy

Could low concentrations of P4 during the estrous cycle following breeding result in poor conception and pregnancy rates? Direct evidence of this is hard to obtain as a developing pregnancy may influence the concentrations of circulating P4. However, plasma P4 concentrations are generally higher during the cycle before breeding in fertile dairy cows than in infertile cows (Folman et al., 1973; Erb et al., 1976; Fonseca et al., 1983). Rosenberg et al. (1977) reported ineffective inseminations were preceded by cycles in which the peak of P4 concentration was reached 8 to 11 d before AI vs the P4 peak being reached 4 to 7 d before AI (i.e., the shape of the P4 curve was important not P4 concentrations per se).

Mean concentrations of P4 were higher in pregnant cows than in cows returning to estrus after breeding (Henricks et al., 1970). Over the first 15 d after mating pregnant heifers had about 1.7 times more P4 in the plasma than those that returned to estrus (Henricks et al., 1971). Progesterone concentrations were lower in infertile cows following AI than in fertile cows (Erb et al., 1976). Concentrations of plasma P4

after AI were higher for Holsteins that conceived compared to those that did not (Fonesca et al., 1983). Beef females with normal developing embryos after AI had higher serum P4 at d 3 to 6 than females with abnormal embryonic development (Maurer and Echternkamp, 1982). It is impossible to say whether the low P4 caused or was a result of the abnormal embryos, but Holstein heifer embryo transfer recipients had lower pregnancy rates when P4 concentrations were low than when concentrations were high at time of transfer (Remsen and Roussel, 1982). In contrast, Sreenan and Diskin (1983) found no difference in P4 concentrations in heifers pregnant to embryo transfer and nonpregnant heifers until d 16 of the cycle (when the CL begins to regress in non pregnant animals).

It would seem possible from the aforementioned data that low P4 concentrations may influence conception and that use of $PGF2\alpha$ (or its synthetic analogs) may precipitate diminished pregnancy rates in Brahman or Brahman crossbred females.

EXPERIMENTAL PROCEDURE

General Procedure

In trial 1, trial 2, and trial 4, purebred Brahman heifers from 24 to 27 months of age were randomly assigned to treatment with a natural $PGF2\alpha$[1] or used as untreated controls. In trial 3, the research population consisted of 22 2-year-old, six 3-year-old, and five 4-year-old purebred Brahman females. In all trials the animals were non-lactating and had displayed at least one estrus prior to initiation of each trial (i.e., they were cycling). Throughout the studies the diet of research animals consisted of coastal bermudagrass hay, molasses-based liquid supplement (16% CP equivalent) and complete mineral mix offered ad libitum plus 1.36 to 1.82 kg (3 to 4 lb) of ground corn per head per day. Overall average daily gain for heifers in the first two trials was .36 kg (.8 lb) (figure 2). Heifers during all trials in the study were considered to be in superior condition. In each trial, all heifers were pastured together and moved as a herd regardless of treatment group. During a 1 month period before initiation of trial 1 and trial 2 heifers were trained to walk through the holding pens and AI chute twice daily (AM and PM) prior to feeding of ground corn. This was to acquaint the heifers with the facility in an effort to minimize stress during the blood collection phase of the trials.

Prostaglandin $F2\alpha$ was administered by intramuscular injection with 3.81 cm (1½ inch) 20 gauge needle into the gluteobiceps. Brahman heifers were monitored for

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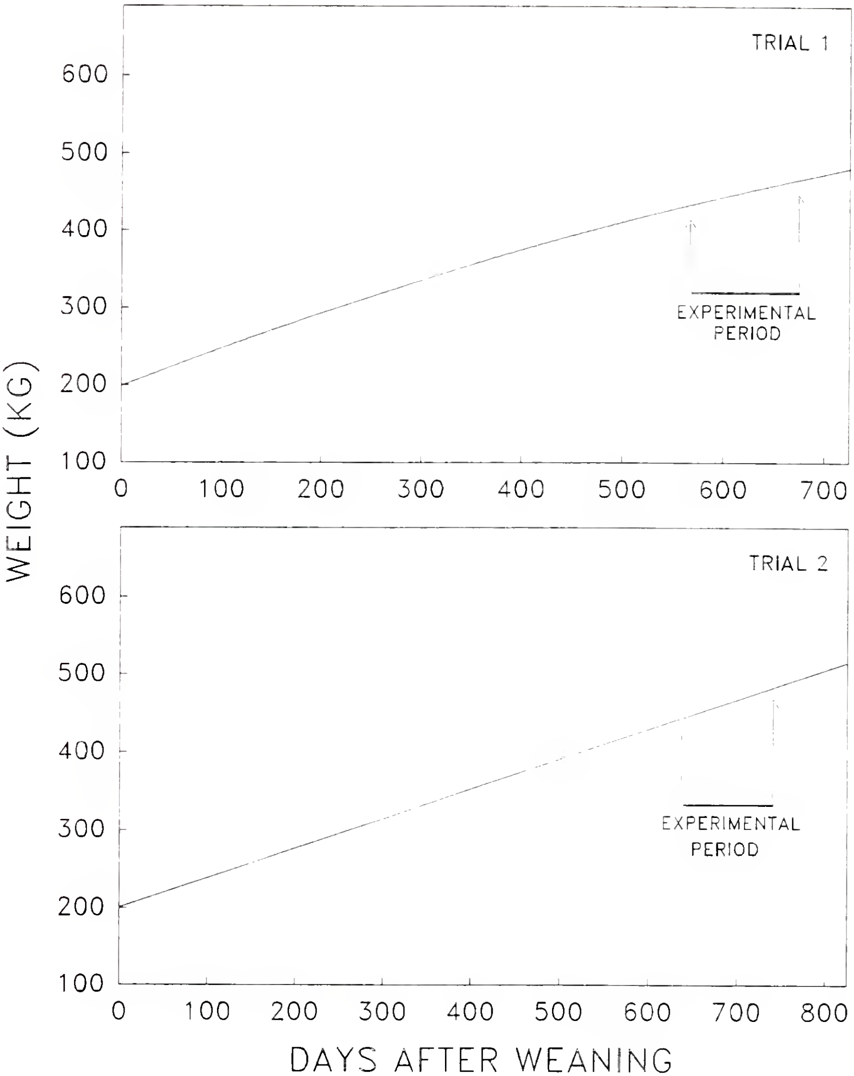
[1]Lutalyse®, UpJohn Co., Kalamazoo, MI.

FIGURE 2. WEIGHT CHANGE FROM WEANING THROUGH EXPERIMENTAL PERIOD FOR HEIFERS IN TRIAL 1 AND TRIAL 2.


TRIAL 1
EXPERIMENTAL PERIOD
600
500
400
300
200
100
0
100
200
300
400
500
600
700
WEIGHT (KG)
TRIAL 2
EXPERIMENTAL PERIOD
600
500
400
300
200
100
0
100
200
300
400
500
600
700
800
DAYS AFTER WEANING

estrus using teaser bulls (surgically deviated penis) equipped with chin ball markers. Records were made of time of day estrus was first observed and circumstance of estrual behavior determination (stood to be mounted by bull, by other heifers, or no longer standing to be mounted but previously marked by bull).

Blood was collected in heparinized Vacutainer tubes (Becton Dickson and Company, Rutherford, NJ) by coccygeal venipuncture and immediately placed in ice water until processed to yield plasma. Plasma was stored at -20°C until assayed for P4.

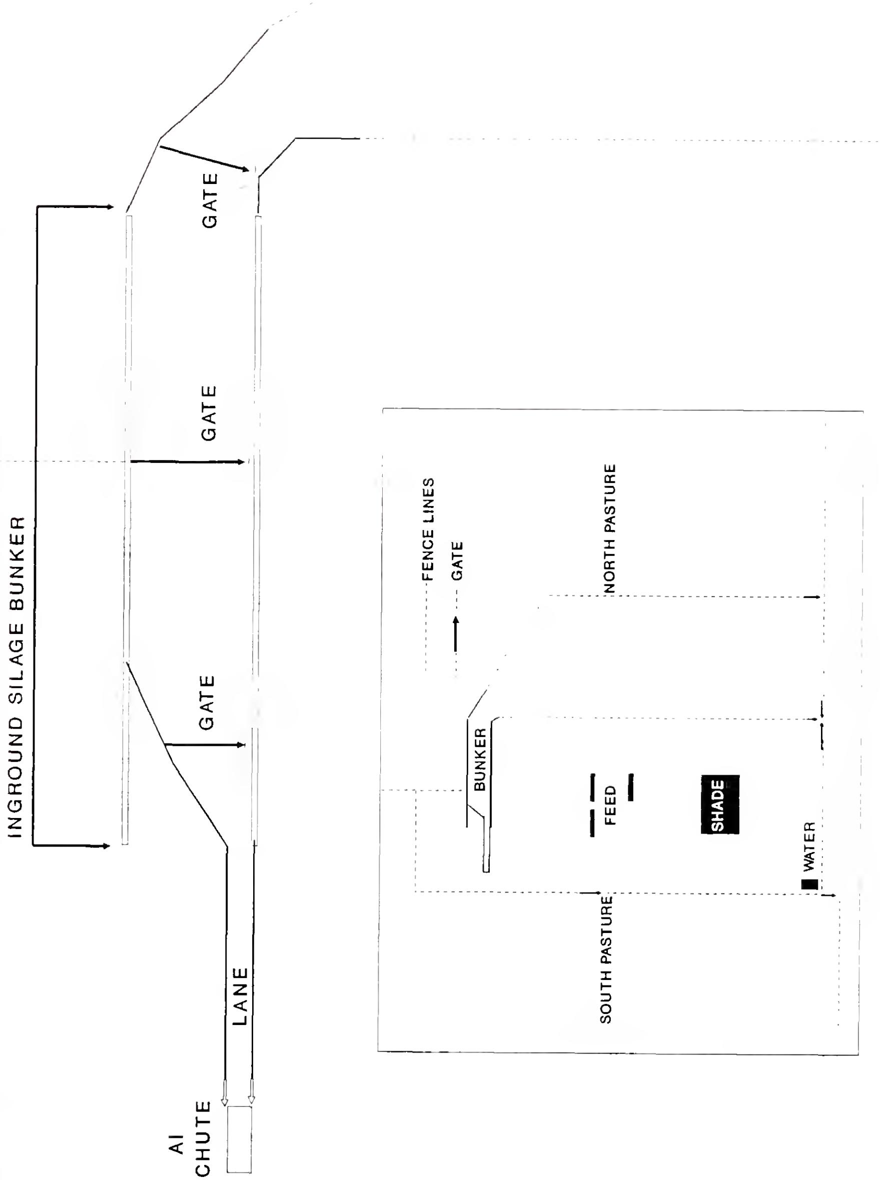
Cattle Handling Facilities

All experiments in this study were conducted at the Purebred Beef Unit (Sandhill) of the Animal Science department, University of Florida, Gainesville. An animal handling facility was constructed using an in-ground concrete silage bunker as the primary corral area (figure 3). Heifers were rotated between the north and south pastures, depending on the available forage. This facility was used strictly for administering PGF2α injections, blood collection and AI of the heifers following treatment. Any other routine handling of the cattle, such as administering of antihelmintic medication or vaccinations, was conducted at a separate corral area. Again, this was to minimize the stress associated with the facility used for treatment and AI.

Radioimmunoassay for Plasma Progesterone

Trial 1. Plasma P4 was determined according to the radioimmunoassay procedure of Abraham et al. (1971) as modified for this laboratory by Lopez-Barbella et al. (1979). An aliquot of sample plasma (500 μl) was placed in a screw top glass tube and spiked with 100 μl of 1000 cpm [1,2-3H]-P4 (New England Nuclear, Boston,

FIGURE 3. CATTLE HANDLING FACILITIES WITH CORRAL CONSTRUCTED FROM IN-GROUND SILAGE BUNKER.


INGROUND SILAGE BUNKER
GATE
GATE
GATE
LANE
AI
CHUTE
FENCE LINES
GATE
NORTH PASTURE
BUNKER
FEED
SHADE
WATER
SOUTH PASTURE

MA.,SA = 53.4 Ci/mmol) in .1 M phosphate buffered saline with .1% gelatin (PBSG). The tube was vortexed and 10 ml ethyl ether was added. The solution was revortexed and the tube plunged into liguid nitrogen for a period of time sufficient to freeze the plasma to a pellet. The ether was decanted into 16 x 100 mm borosilicate tubes and evaporated under nitrogen gas. Five milliliters of PBSG was added to the tube and vortexed to resusupend the dried ether extract of the plasma sample.

Progesterone antiserum, provided by Dr. L. Fleeger of Texas A & M University, College Station, was developed in a rabbit against P4 conjugated to bovine serum albumin (BSA). Progesterone concentrations were determined against a linear standard curve of P4 from 1000 to 31.25 pg/ml using the procedure described in Appendix A.

This assay was validated by adding to five replicates 1000, 500, 250, 125, 61.5, and 31.25 pg P4/ml plasma (from an ovariectomized cow). A linear regression equation of added vs measured P4 described differences among concentrations [Y = 2.66 + 1.05X; Y = amount of P4 measured (pg/ml) and X = amount of P4 added (pg/ml); R^2 = .98]. Recovery of the extracted P4 spiked samples was 90%. Intra- and inter-assay coefficients of variation for sample assays, determined by assay of standard plasma collected during diestrus, were 4.3% and 10.0%, respectively.

Trial 2. Plasma P4 concentrations were determined by a procedure similar to the one outlined for trial 1. Progesterone antiserum for these assays was provided by Dr. Juan Troconiz and Dr. Megalay de Manzo from the Universidad Central Venezuela, at Maracay, Venezuela. This antiserum was generated in sheep against P4 conjugated to BSA. In this procedure an aliquot of sample plasma (500 μl) was extracted with 5 ml benzene and hexane (1:2), frozen, and the solvent decanted (Louis et al., 1973). The decanted solvent was then evaporated under nitrogen gas and the assay

proceeded as described above. Validation for this assay was conducted as for the previous assay and a linear regression equation described differences among concentrations [Y = 2.63 + 1.01X; Y = amount of P4 measured (pg/ml) and X = amount of P4 added (pg/ml); R^2 = .93]. Recovery of the extracted P4 spiked samples was 97%. Intra- and inter-assay coefficients of variation for the sample assays, determined by assay of standard plasma collected during diestrus, were 8.9% and 14.6%, respectively.

Experimental Protocol

Trial 1. Prior to trial 1, all heifers were monitored for estrus and then bled daily from d 2 to d 14 of the first spontaneously occurring estrous cycle after March 1. This established a base of data demonstrating the plasma P4 concentrations of normally cycling Brahman heifers during the luteal phase of the estrous cycle.

After this initial blood collection period, trial 1 was initiated to determine whether the PGF2α induced CL was different from the spontaneously occurring CL in Brahman heifers in terms of plasma concentrations of P4. Treatment heifers were not synchronized as a group but treated were individually and injected intramuscularly either once or twice (with 11 d interval between injections) with 25 mg PGF2α. Heifers in the control group received no injection but were handled in the same manner as treated heifers. The first injection was given on either d 7 or d 14 of the estrous cycle (estrus = d 0) and blood was collected once daily (0700 h) on d 2 to d 14 of the induced or naturally occurring estrous cycle (table 1). If a heifer did not express estrus after treatment with PGF2α the bleeding regimen was started on d 6 after the final injection and continued for 13 d. Artificial insemination was delayed to 12 h (AM/PM

TABLE 1. EXPERIMENTAL DESIGN FOR TRIAL 1: TO DETERMINE IF THE PGF2α INDUCED CL PRODUCES LOWER CONCENTRATIONS OF PLASMA P4 THAN THE SPONTANEOUSLY OCCURRING CL

Group	No. of heifers	Treatment[a]	Day of cycle at first PGF2α injection	Estrous cycle of bleed (d 2 to 14)
C	8	no PGF2α	---	1st cycle
1A	6	1 x PGF2α	7	1st cycle post PGF2α
1B	6	1 x PGF2α	14	1st cycle post PGF2α
2A	6	2 x PGF2α	7	1st cycle post 2nd PGF2α injection
2B	6	2 x PGF2α	14	1st cycle post 2nd PGF2α injection

[a] Heifers were injected with 25 mg PGF2α intramuscularly either once or twice with the second injection given 11 d after the first.

rule) after the first naturally occurring estrus following blood sample collection to avoid pregnancy confounding the P4 data.

Trial 2. A second experiment was devised to further evaluate the effect of day of cycle when PGF2α is administered on expression of estrus. Plasma P4 was monitored in an attempt to elucidate PGF2α effect in non-responding heifers. Brahman heifers were randomly assigned to treatment as shown in table 2.

Heifers were treated individually with 25 mg PGF2α and not synchronized as a group. They were bled twice daily (0700 h and 1900 h) from 1 d before injection to 3 d after the induced estrus (or from d 16 of the natural cycle in the case of untreated animals). If a heifer failed to express estrus following an injection, she was bled twice daily until 6 days after injection. Control heifers received no injections but were moved through the corral and chute with the treated heifers at the time of injection and blood collection. Heifers in this trial were AI at the PGF2α induced estrus.

Trial 3. A third trial was conducted to determine if two injections of PGF2α given 24 h apart would induce estrus more effectively than a single injection. Non-lactating Brahman heifers and cows were monitored for estrus and then assigned to one of two treatment groups. Heifers were given either a single intramuscular injection of 25 mg PGF2α on d 7 of the estrous cycle or two 25 mg injections with the first on d 7 and the second on d 8 of the cycle. A split plot design was used and each heifer was treated twice (phase 1 and phase 2) during the study (table 3). Animals were AI 12 h after the onset of the last induced estrus (AM/PM rule) following treatment in the second phase of the study.

Trial 4. In the fourth year of this study a preliminary trial was conducted to assess the possibility of incorporation of double injections at a 24 h interval into the

TABLE 2. EXPERIMENTAL DESIGN FOR TRIAL 2: TO FURTHER EVALUATE THE EFFECT OF DAY OF CYCLE ON WHICH PGF2α IS GIVEN ON THE EXPRESSION OF ESTRUS

Group	No. of heifers	Treatment[a]	Day of cycle at PGF2α injection	Time of bleeding
1	6	no PGF2α	---	d 16 to estrus + 3 d
2	6	1 x PGF2α	7	d 6 to estrus + 3 d
3	6	1 x PGF2α	10	d 9 to estrus + 3 d
4	6	1 x PGF2α	14	d 13 to estrus + 3 d
5	6	1 x PGF2α	18	d 17 to estrus + 3 d

[a] Heifers were injected once with 25 mg PGF2α given intramuscularly.

TABLE 3. EXPERIMENTAL DESIGN FOR TRIAL 3: TO DETERMINE IF TWO INJECTIONS OF PGF2α GIVEN 24 HOURS APART INDUCE ESTRUS MORE EFFECTIVELY THAN A SINGLE INJECTION

Phase		No. of heifers	Treatment[a]	Day of cycle at PGF2α injection
1		16	1 x PGF2α	d 7
		17	2 x PGF2α	d 7 and d 8
2	no. PGF2α injections in Phase 1			
	1	8	1 x PGF2α	d 7
	2	8	1 x PGF2α	d 7
	1	8	2 x PGF2α	d 7 and d 8
	2	9	2 x PGF2α	d 7 and d 8

[a] Heifers were injected with 25 mg PGF2α intramuscularly either once or twice with the second injection given 24 h after the first.

traditional PGF2α management protocol (two injections at an 11 d interval). To this end, 23 Brahman heifers were monitored for estrus and then treated, as a group, with two injections of 25 mg PGF2α given 24 h apart. Stage of estrous cycle at time of injection was recorded for each animal. Fertile Brahman bulls equipped with chin ball markers were placed with the heifers at time of PGF2α treatment, making time of estrus also the time of breeding in this trial. Pregnancy was determined by rectal palpation at 65 d after PGF2α treatment.

Statistical Analysis of Data

Plasma P4 data from trial 1 and trial 2 were analyzed using the least squares analysis of variance and polynomial regression of the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 1985). Data in trial 1 were analyzed by comparing a model consisting of treatment, response, treatment by response, animal within treatment by response, and day up to the third order as sources of variation with models in which the day variable was replaced with either day by treatment or day by response to the third order. Possible differences in regression relationships due to treatment and response were tested by examining the heterogeneity of slopes (appendix tables 11, 12, and 13). Plasma P4 data in trial 2 were analyzed in a similar manner with the model consisting of treatment, response, treatment by response, animal within treatment by response, and period to the third order as sources of variation (appendix tables 15, 16, and 17).

The Catmod (Chi-square analysis) procedure of the SAS (1985) was used to compare the effect of treatment and day of treatment on rates of estrual response in trials 1, 2, and 3 (appendix tables 14 and 18). Chi-square analysis was also used to determine if there was a difference in degree of synchrony of estrual response

following treatment with a single 25 mg injection of PGF2α on d 7 of the estrous cycle as compared to a series of two 25 mg injections of PGF2α with the first given on d 7 and the second given on d 8 (trial 3, appendix table 19). Two-sided t-tests were used to test for a possible effect of treatment on interval from PGF2α injection to induced estrus (appendix table 20).

RESULTS AND DISCUSSION

Trial 1

The administration of either a single injection or a series of two injections (the second given 11 d after the first) of a natural PGF2α (25 mg) did not adversely affect the plasma P4 concentrations on d 2 to d 14 of the induced estrous cycle indicating formation of a normal functioning CL after treatment (figure 5). Heifers given a single injection of PGF2α on d 7 of the cycle (treatment 1A) had slightly higher P4 concentrations than heifers in the control group (treatment C, $P<.01$). This was largely due to the influence of heifer #28. Because there were two nonresponders in this group the third order regression curve was based on the data from only four heifers. Heifer #28 had plasma P4 concentrations much higher than the other three heifers in this treatment group with a peak of 13.04 ng/ml on d 12 of the estrous cycle. The mean P4 concentration for the other three heifers on the same day the cycle was 7.78 $\pm$ 1.31 ng/ml (mean $\pm$ SE). Progesterone concentrations in this same heifer on d 12 during the estrous cycle before treatment (appendix table 6) peaked at 12.84 ng/ml while P4 at the same time for the other three heifers was 7.24 $\pm$ 1.43 ng/ml. Although the third order regression curve for treatment 1A was not parallel with the curve for the controls, it was concluded the difference was not due to PGF2α treatment. For all other treatments plasma P4 concentrations during an induced estrous cycle were similar to those of the controls and to those of all heifers during the estrous cycle prior to treatment with PGF2α (figures 4 and 5 and table 4).

FIGURE 4. PLASMA P4 PROFILES FROM D 2 TO D 14 OF THE ESTROUS CYCLE PRIOR TO $PGF_{2\alpha}$ TREATMENT FOR ALL HEIFERS IN TRIAL 1 (BY TREATMENT).

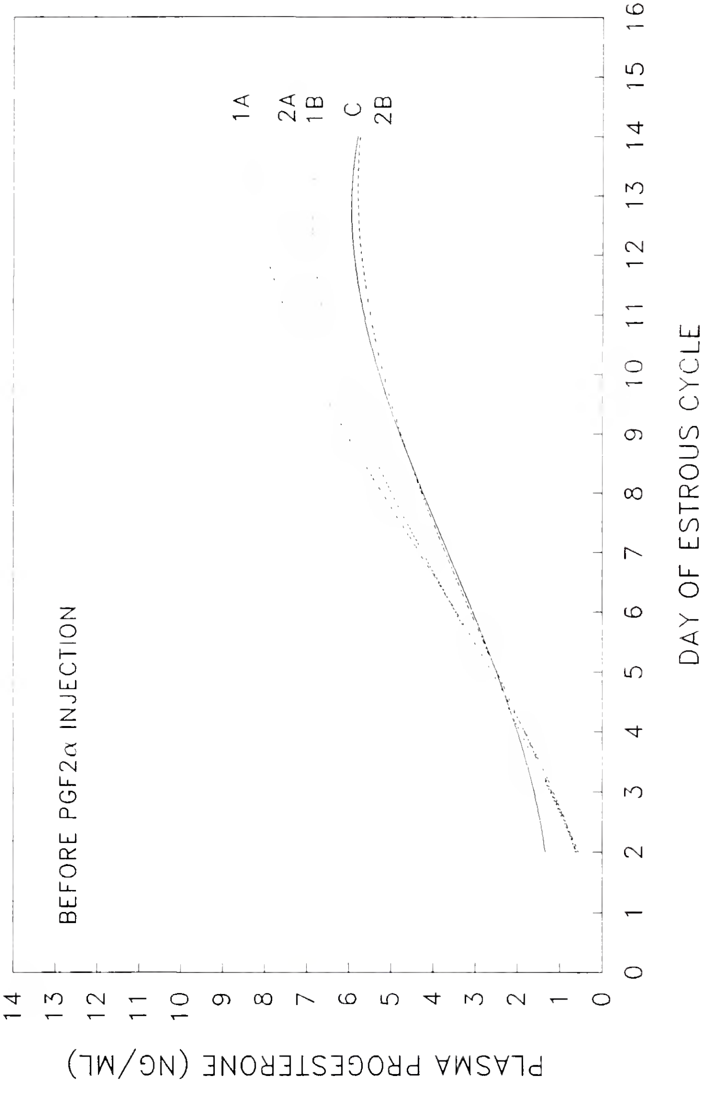

BEFORE PGF2α INJECTION
1A
2A
1B
C
2B
DAY OF ESTROUS CYCLE
PLASMA PROGESTERONE (NG/ML)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIGURE 5. PLASMA P4 PROFILES FROM D 2 TO D 14 OF THE INDUCED AND CONTROL ESTROUS CYCLES AFTER PGF2α TREATMENT FOR TRIAL 1 (BY TREATMENT).

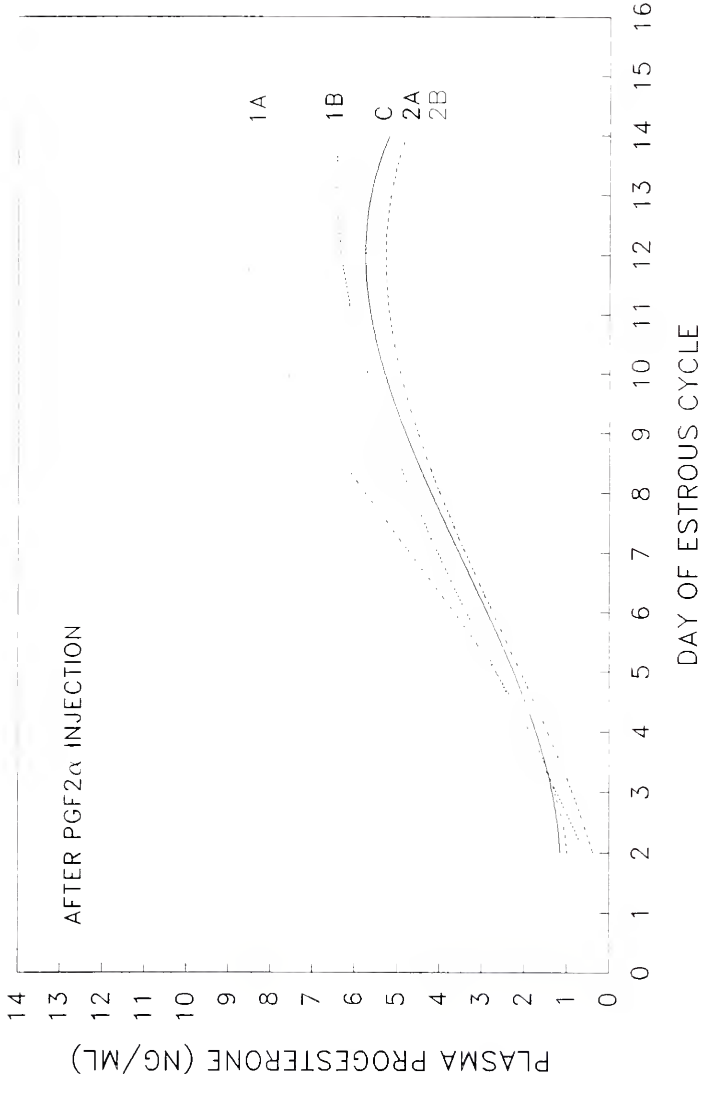

AFTER PGF2α INJECTION
1A
1B
C
2A
2B
PLASMA PROGESTERONE (NG/ML)
DAY OF ESTROUS CYCLE
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

TABLE 4. MEANS OF PLASMA P4 CONCENTRATIONS (NG/ML) ON DAYS 2 TO 14 OF THE ESTROUS CYCLE BEFORE AND AFTER PGF2α INJECTION (TRIAL 1)

	Day of Estrous Cycle												
Cycle	2	3	4	5	6	7	8	9	10	11	12	13	14
Pre-PGF2α	.89 ± .12[a]	1.22 ± .17	1.65 ± .18	2.73 ± .25	3.59 ± .22	4.21 ± .26	4.85 ± .31	5.37 ± .28	6.09 ± .30	6.53 ± .35	6.46 ± .41	6.79 ± .36	6.82 ± .41
Post-PGF2α (responders)	1.18 ± .33	1.39 ± .39	1.94 ± .63	2.68 ± .33	3.50 ± .28	4.37 ± .35	4.82 ± .49	5.77 ± 31	5.79 ± .34	6.30 ± .47	6.67 ± .56	6.21 ± .44	6.20 ± .58

[a]Values are mean ± SE

Jiménez et al. (1985) also reported no difference in P4 concentrations of Brown Swiss or Indubrazil cows before and after treatment with a natural PGF2α.

In contrast, other researchers have reported that Brahman cows and heifers treated with the PGF2α analog cloprostenol had lower serum P4 concentrations on d 2 to 13 of the induced estrous cycle than in naturally occurring estrous cycles. In addition, treatment with cloprostenol on d 8 to 12 of the estrous cycle resulted in development of a smaller CL which contained lower concentrations of P4 (Hardin and Randel, 1982). Similarly, use of the PGF2α analog alfaprostol in Brahman heifers resulted in lower P4 concentrations during the induced estrous cycle and produced a CL with fewer small and large luteal cells which had lower in vitro P4 production in response to LH challenge when compared to CL formed following spontaneous estrus (Hansen et al, 1987b).

One explanation for the lower P4 concentrations reported in the previously mentioned studies might be the effect of stress induced by the intensive handling of the research animals necessary for blood sample collection. While the effects of stress on P4 concentrations is poorly documented it has been suggested stress elicits a release of corticosteroids from the adrenal glands which in turn results in an increase in P4 release from the adrenals (Wagner et al., 1972). Holstein heifers that were stressed had increased corticosteroid concentrations (Stoebel and Moberg, 1982a). Administration of adrenocorticotropin hormone (ACTH) on d 1 to 8 of the estrous cycle in heifers produced elevated corticosteroid concentrations as well as transient increases in plasma P4 on d 1 to 5 followed by a significant decrease in P4 concentrations on d 8 to 10 (Wagner et al., 1972). The researchers suggested the increase in P4 concentrations was due to secretion of corticosteroids by the adrenals and that the

subsequent decrease in plasma P4 was due to a negative feedback on the hypothalamus or pituitary which might have been sufficient to block normal LH production. Stoebel and Moberg (1982b) reported use of ACTH caused increased P4 secretion by the adrenal cortex which resulted in elevated plasma P4 concentrations in dairy cows. Heat stress of cows caused lower basal and peak LH concentrations (Madan and Johnson, 1973). Stressed heifers had no LH surge following estrus but unstressed heifers did (Stoebel and Moberg, 1982a). Hardin and Randel (1982) reported the handling of Brahman females prior to estrus had detrimental effects on the endocrine changes during the periestrous period but that frequent sampling during the luteal phase did not alter the reproductive cycle. In the study presented here much effort was exerted to minimize the amount of stress imposed on the research animals (through training, facility use, and method of blood collection). It is believed that the effects of stress on estrual response and P4 concentrations were negligible. During these trials all heifers (treated and controls) were handled in exactly the same manner. Presumably, if there was an effect of stress present it influenced all treatment groups equally.

Another explanation for dissimilarities in the previously mentioned studies (Hardin and Randel, 1982; Hansen et al., 1987b) and the data presented here is the possibility that use of the $PGF2\alpha$ analogs had an adverse effect on the subsequently forming CL. Hansen et al. (1987b) suggested artificial shortening of the estrous cycle may alter selection of the ovulatory follicle and differentiation of the granulosa and theca interna cells to luteal cells which might result in the formation of a subfunctional CL. Since treatment with a natural $PGF2\alpha$ did not result in lower P4 concentrations it

is conceivable the use of these $PGF2\alpha$ analogs, instead of shortening of the cycle per se, could result in lower P4 production.

The intent of this study was to determine whether the $PGF2\alpha$ induced CL produced lower concentrations of plasma P4 than spontaneously occurring CL in Brahman heifers. Days on which $PGF2\alpha$ was to be administered were selected to test response when treated in the early and mid luteal phase. Unexpectedly, only 67% of the heifers injected with $PGF2\alpha$ on d 7 of the cycle expressed estrus within 7 d after injection while 100% of those injected on d 14 exhibited estrus (table 5). This was reflected in the plasma P4 profiles for heifers that expressed estrus within 7 d after treatment (responders) vs those that did not (nonresponders, figure 6; $P<.001$). Blood sample collection was initiated on d 6 after injection in heifers that failed to exhibit estrus. Plasma P4 profiles for two representative animals given a single injection on d 7 are shown in figure 7. Heifer #59 responded to the 25 mg of $PGF2\alpha$ and displayed estrus on the second day following injection. Heifer #72 did not respond to the $PGF2\alpha$ and expressed estrus 10 d after injection (or 17 d after the previous estrus - a normal cycle). Graphs of P4 concentrations for all nonresponders (figure 8) demonstrate the diverse patterns of P4 for these heifers. Heifers P4 profiles were dependent on the length of the individual estrous cycle. Heifer #1 (lower panel), for example, expressed estrus 10 d after the first $PGF2\alpha$ injection (a 17 d estrous cycle) and so received the second injection on d 1 of the estrous cycle. The heifer then exhibited estrus 2 d after the end of the bleeding regimen (a 20 d estrous cycle). Heifer #83 (upper panel) expressed estrus on the third day of the regimen (a 17 d estrous cycle).

Some researchers have reported a lower response rate when heifers are injected with $PGF2\alpha$ or its analogs early in the cycle (Roche, 1974; Macmillan, 1978;

TABLE 5. SYNCHRONIZATION AND PREGNANCY RATES OF PGF2α TREATED AND CONTROL HEIFERS (TRIALS 1, 2, AND 3)

Trial	Trt. group	No. of inj.	Day of cycle at first inj.	Interval between inj.	n	In estrus by 7 d post-1st inj., %	Days from inj. to estrus	In estrus by 7 d post-2nd inj., %	Days from inj. to estrus	Pregnancy to 1st AI %[a]	Pregnancy rate, overall %[b]
1	1A	1	7	--	6	67	2.75	--	--	67	67
	2A	2	7	11 d	6	67	3.25	67	3.25	50	67
	1B	1	14	--	6	100	3.67	--	--	33	50
	2B	2	14	11 d	6	100	4.00	67	2.25	50	50
	C	0	--	--	8	--	--	--	--	50	63
Overall					32	83	3.50	67	2.75	50	60
2	1	1	7		6	50	2.83			50	67
	2	1	10		6	67	4.25			50	67
	3	1	14		6	100	3.25			33	83
	4	1	18		6	100	3.33			33	67
	C	0	--		6	--	--			33	33
Overall					30	79	3.42			40	63
3	1	1	7	--	32	72[c]	3.63[d]			31	67
	2	2	7	24 h	34	97	2.71			38	81
Overall					66	85	3.09			33	74

[a] Heifers in trial 1 were AI at the first non-induced estrus following last PGF2α injection to avoid pregnancy confounding the P4 data. Heifers in trials 2 and 3 were AI at the last induced estrus. Only 4/6 heifers in treatment group 2B in trial 1 were inseminated.

[b] Overall pregnancy rates in trials 1 and 2 resulted from multiple AI after treatment followed by natural breeding by clean-up bull. In trial 3, overall pregnancy rate was the result of a single AI followed by natural breeding.

[c] Chi-square analysis indicates treatments differ within trial 3, $P<.02$

[d] Two-sided t-test indicates treatments differ within trial 3, $P<.01$

FIGURE 6. PLASMA P4 PROFILES FROM D 2 TO D 14 OF THE INDUCED ESTROUS CYCLE FOR HEIFERS THAT RESPONDED TO PGF2α TREATMENT AND FROM D 6 TO D 18 AFTER THE PGF2α INJECTION FOR NONRESPONDING HEIFERS (TRIAL 1).

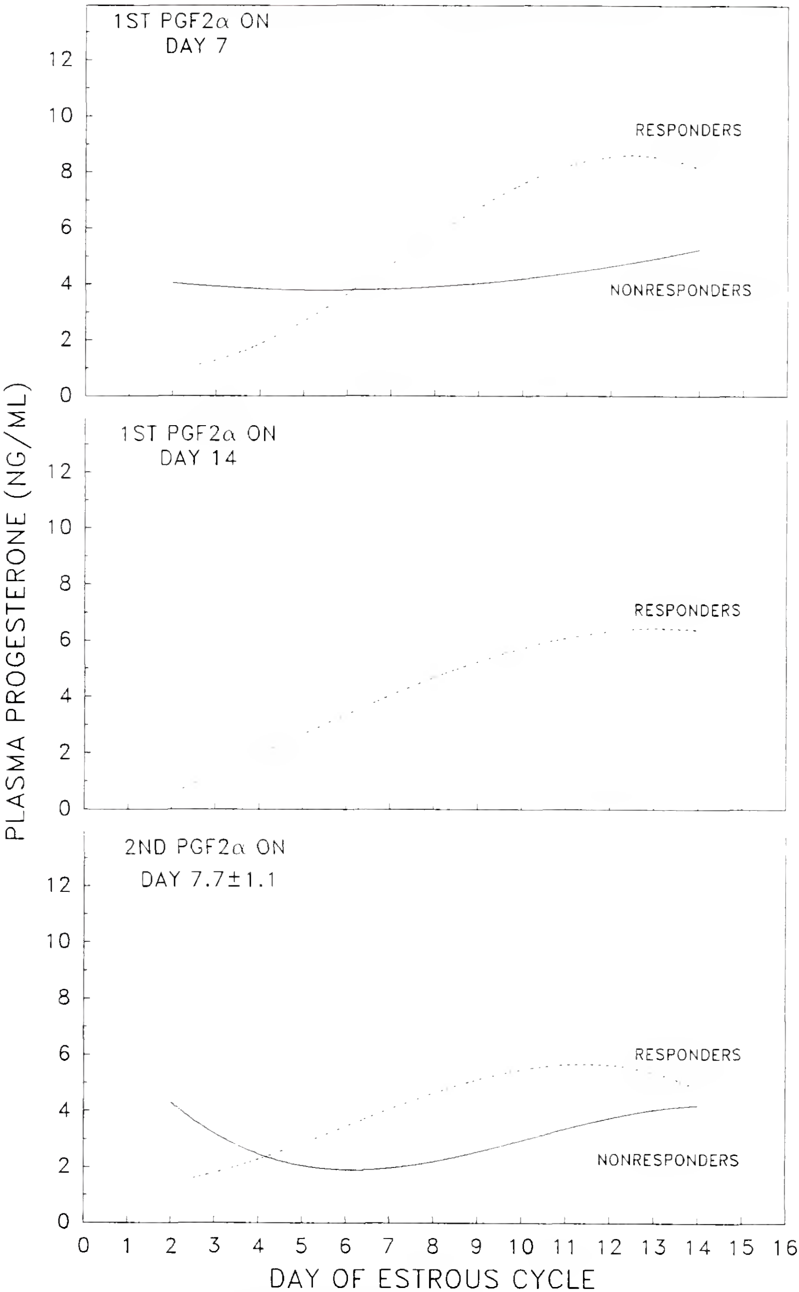

1ST PGF2α ON
DAY 7
RESPONDERS
NONRESPONDERS
1ST PGF2α ON
DAY 14
RESPONDERS
2ND PGF2α ON
DAY 7.7±1.1
RESPONDERS
NONRESPONDERS
PLASMA PROGESTERONE (NG/ML)
DAY OF ESTROUS CYCLE
0
2
4
6
8
10
12
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

FIGURE 7. P4 CONCENTRATIONS FOR HEIFER #59 (RESPONDER) AND HEIFER #72 (NONRESPONDER) IN TRIAL 1.

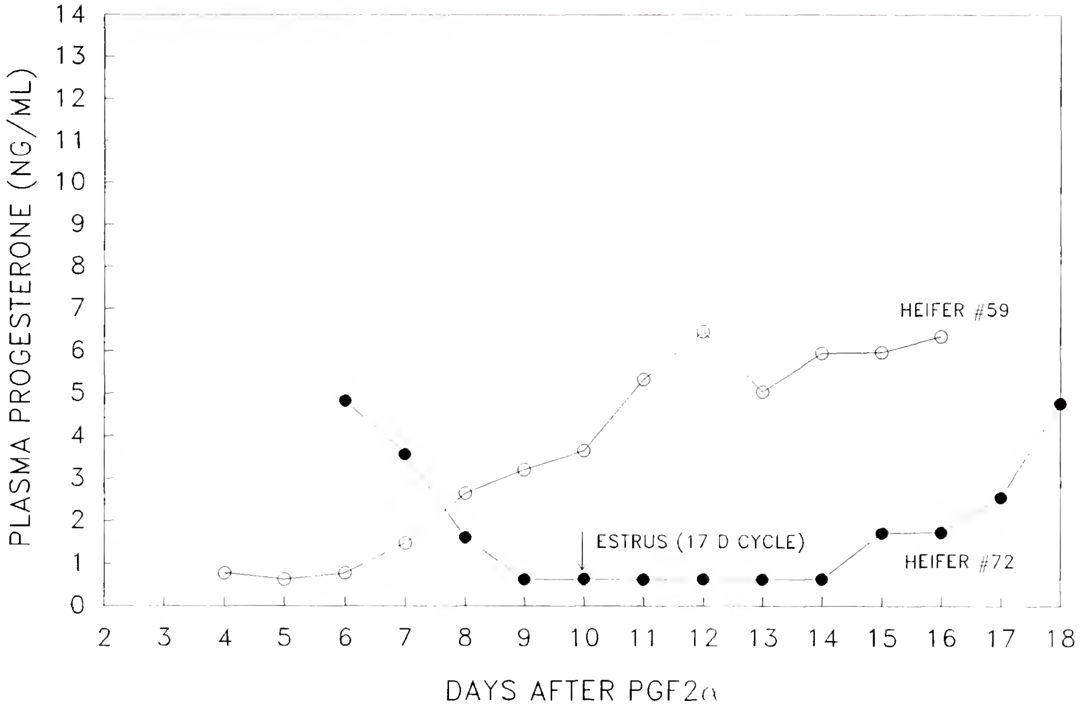
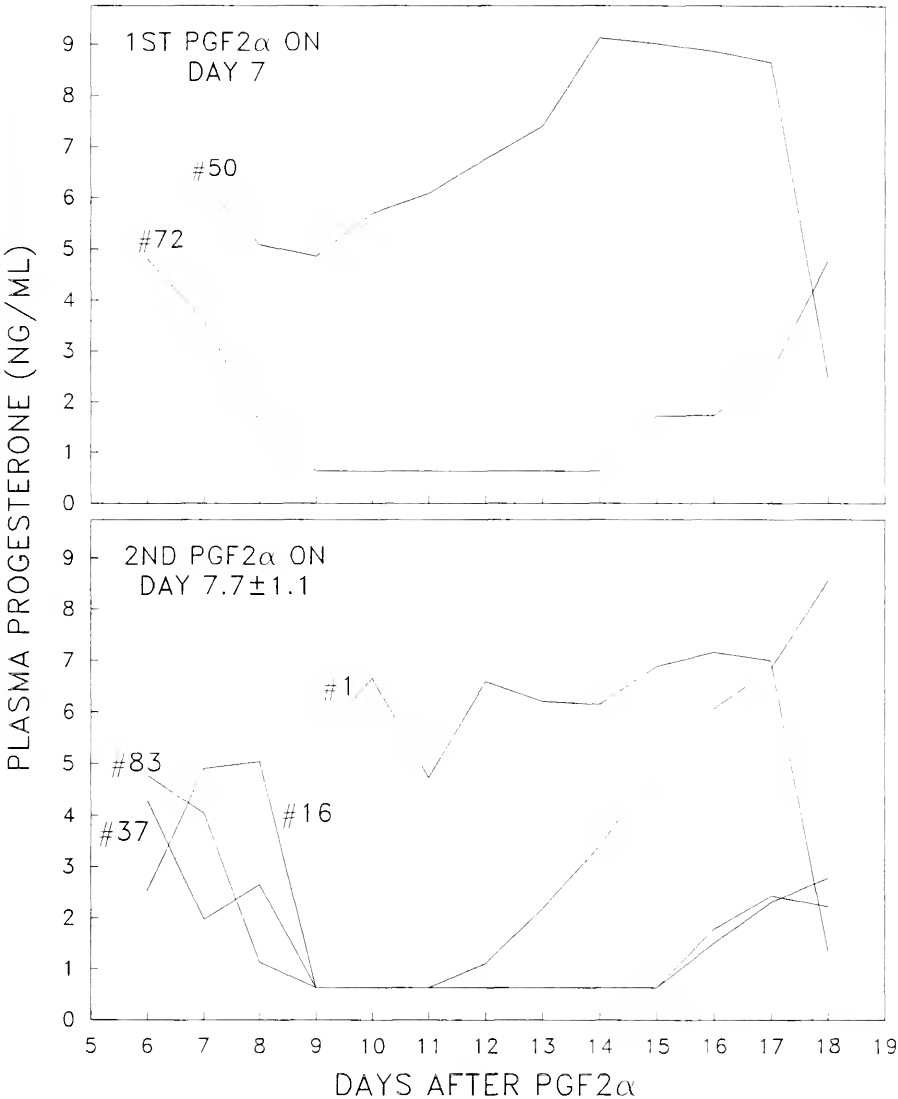

PLASMA PROGESTERONE (NG/ML)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
DAYS AFTER PGF2α
HEIFER #59
HEIFER #72
ESTRUS (17 D CYCLE)

FIGURE 8. PLASMA CONCENTRATIONS OF P4 FOR ALL NONRESPONDING HEIFERS IN TRIAL 1.



1ST PGF2α ON
DAY 7
#50
#72
2ND PGF2α ON
DAY 7.7±1.1
#1
#83
#37
#16
PLASMA PROGESTERONE (NG/ML)
DAYS AFTER PGF2α

King et al., 1982; Macmillan, 1983; Wahome et al., 1985; Watts and Fuquay, 1985) while others reported good response to treatment at this time (Edqvist et al., 1975; Gonzalez et al., 1985). The failure of PGF2α treatment to induce estrus in all heifers when injected on d 7 is especially troublesome when the two injection system of PGF2α synchronization (second injection given 10 to 12 d after the first, Roche; 1974) is used. In trial 1, heifers that were given a first PGF2α injection on d 7 or d 14 of the estrous cycle followed by a second 11 d later demonstrated estrus 67% of the time following the second injection (table 5). In view of the low response rate to a first or only injection on d 7 of the cycle, this would not be unexpected as heifers that responded to the first injection on d 7 or d 14 were at 7.75 d and 7.00 d, respectively, of the induced estrous cycle at the time of the second injection (table 6). Indeed, this system of PGF2α synchronization depends on a majority of animals being at d 7 to 8 of the estrous cycle at the time of the second injection.

Artificial insemination was postponed in this trial until the first naturally occurring estrus after final PGF2α injection to avoid pregnancy confounding the P4 data. There was an overall pregnancy rate of 60% with a first service pregnancy rate range of 33 to 67% (table 5). Trial 1 was designed to examine the effect of PGF2α on plasma progesterone concentration and so the number of heifers in each treatment group were limited. The small number of heifers in each treatment group did not allow a valid statistical analysis of the response or pregnancy data.

Trial 2

A second trial was conducted to further evaluate the effect of day of cycle when PGF2α is administered on expression of estrus. In trial 2, as in previous trial, the rate of estrous response differed with day of injection (table 5). Only 50% of the heifers

TABLE 6. MEAN DAY OF ESTROUS CYCLE AT THE TIME OF SECOND PGF2α INJECTION (TRIAL 1)

Day of cycle at 1st injection	Day of cycle at 2nd injection	Range
7	7.75	7 - 9
14	7.00	5 - 9

injected on d 7 of the estrous cycle and 67% injected on d 10 expressed estrus within 7 d following treatment with PGF2α. All heifers injected on d 14 responded to the PGF2α. Likewise, all heifers injected on d 18 of the cycle expressed estrus within 7 d. When data from trials 1 and 2 were combined, significantly fewer heifers expressed estrus after the first or only PGF2α injection on d 7 (61%) than those given a first or only injection on d 14 (100%) ($P<.05$; table 7).

In this same combined data set the interval from PGF2α to estrus tended to be shorter for heifers injected on d 7 than for heifers injected on d 14 (2.95 d vs 3.64 d, respectively; $P<.09$; table 7). Similar findings for either natural PGF2α or PGF2α analogs were reported by other researchers (Jackson et al., 1979; Refsal and Seguin, 1980; King et al., 1982; Stevenson et al., 1984). Jackson et al. (1979) suggested the shorter interval to estrus when PGF2α is injected early in the cycle may be attributed to an early wave of follicular growth and the resultant increase in plasma estrogen. Pierson and Ginther (1984), using ultrasonography, determined there were two follicular waves during the estrous cycle of the cow with the first large follicle in the first wave regressing around mid-cycle. Sirois and Fortune (1988), however, indicated the ultrasonography of the ovaries in heifers showed three waves of follicular development with the first beginning on d 1.9, the second on d 9.4, and the third on d 16.1. The effect that developing follicles may have on interval to estrus following PGF2α treatment is probably due to the peaks of estrogen which follow the same wavelike pattern of follicular growth (Hansel and Echternkamp, 1972; Shemesh et al., 1972; Dobson and Dean, 1974; Glencross and Pope, 1981). As previously discussed estrogen, OT and PGF2α act in concert to effect luteolysis. High plasma concentrations of estrogen may act to drive the luteal regression initiated by a PGF2α injection.

TABLE 7. SYNCHRONIZATION RATES AND INTERVAL FROM INJECTION TO ESTRUS ON D 7 OR D 14 OF THE ESTROUS CYCLE (TRIALS 1 AND 2 COMBINED)

Day of cycle at 1st injection	Number of heifers	In estrus by 7 d post-inj., %	Days from injection to estrus ± SE
7	18	61	2.95 ± .41
14	18	100*	3.64+ ± .24

* P<.05

+ P<.09

Heifers in this trial were AI to the induced estrus after PGF2α (AM/PM rule). There was an overall pregnancy rate of 63% with a first service pregnancy rate range of 33 to 50%. As in trial 1, the purpose of trial 2 was to examine the effect of PGF2α on P4 concentrations. The small number of heifers in each treatment group precluded valid statistical analysis of the effect of treatment on response and pregnancy rate within trial 2.

Plasma P4 was measured in trial 2 as an attempt to further elucidate PGF2α effect in nonresponding heifers. Regression curves of plasma P4 concentrations, from time of PGF2α injection, for responding and control heifers are shown in figure 9. Progesterone profiles from the time of injection differed due to treatment with untreated heifers in the control group having a slower rate of P4 decline ($P<.01$). Figure 10 shows the means $\pm$ SE of plasma P4 concentrations for heifers that either expressed estrus within 7 d after PGF2α (responders) or did not (nonresponders). All treated heifers demonstrated a precipitous decline in plasma P4 by 12 h after injection and P4 continued to decline until 24 h after injection. The depressed concentrations of P4, however, began to increase within 48 h after PGF2α in the heifers that failed to express estrus after treatment. Analysis of the third order regression curves for these data indicate plasma P4 concentrations for nonresponders continued to increase ($P<.001$) and reached concentrations approximately three times greater than in heifers exhibiting estrus by 6 d after PGF2α injection. The drop in plasma P4 by 12 h after injection and the subsequent increase is common to all animals in trial 2 that failed to express estrus after treatment (figure 11). Plasma P4 concentrations for nonresponders following a PGF2α injection on d 7 (upper panel) and d 10 (lower panel) are shown in figure 11.

FIGURE 9. PLASMA P4 PROFILES FROM $PGF_{2\alpha}$ INJECTION FOR ALL INDUCED AND CONTROL ESTROUS CYCLES IN TRIAL 2 (BY TREATMENT).

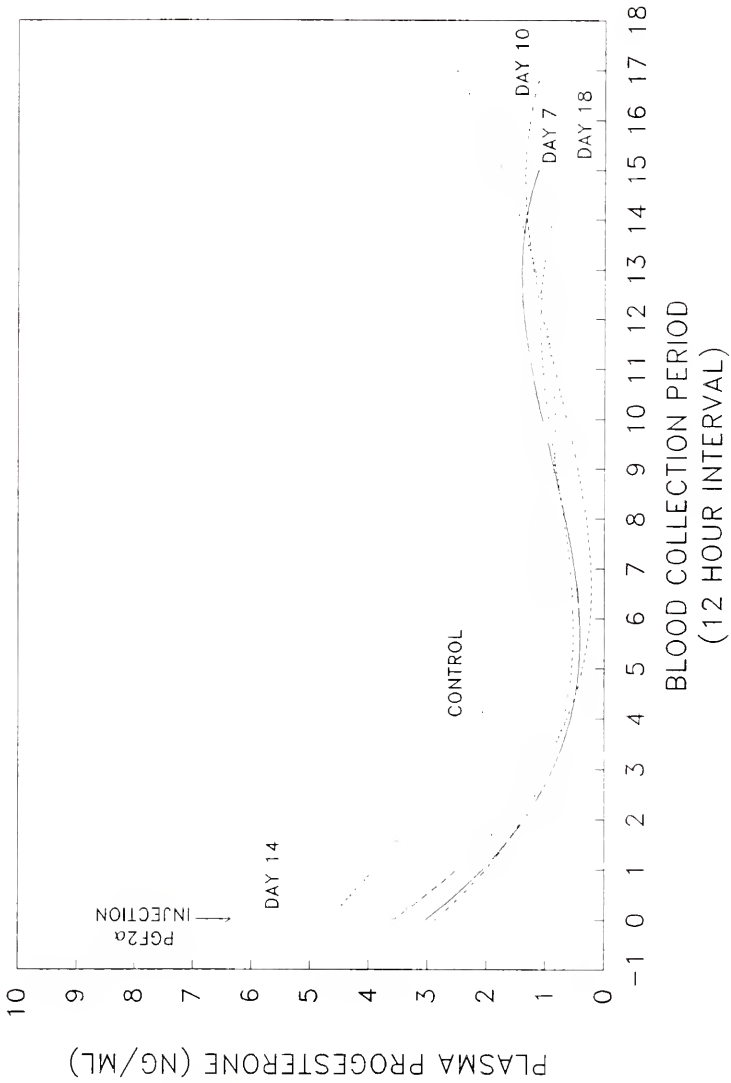

PLASMA PROGESTERONE (NG/ML)
BLOOD COLLECTION PERIOD
(12 HOUR INTERVAL)
PGF2α INJECTION
DAY 14
CONTROL
DAY 10
DAY 7
DAY 18

FIGURE 10. PLASMA P4 CONCENTRATIONS (MEANS ± SE) FROM 1 D BEFORE PGF2α INJECTION FOR ALL RESPONDING AND NONRESPONDING HEIFERS IN TRIAL 2.

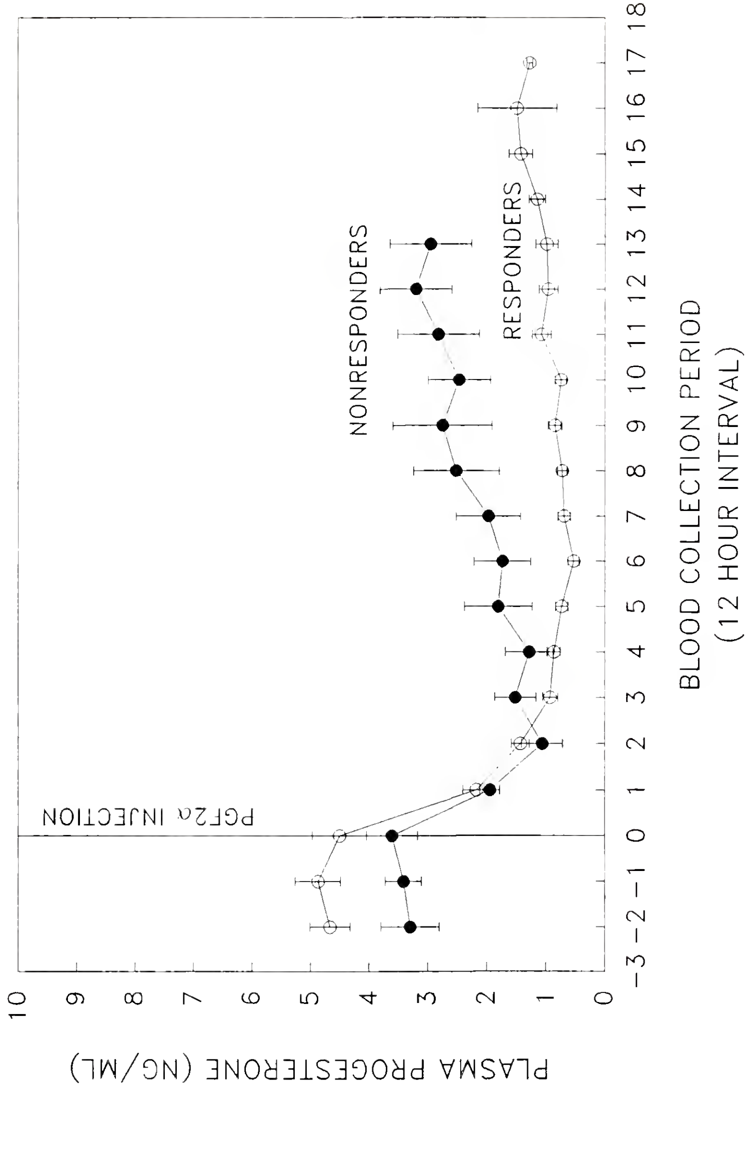
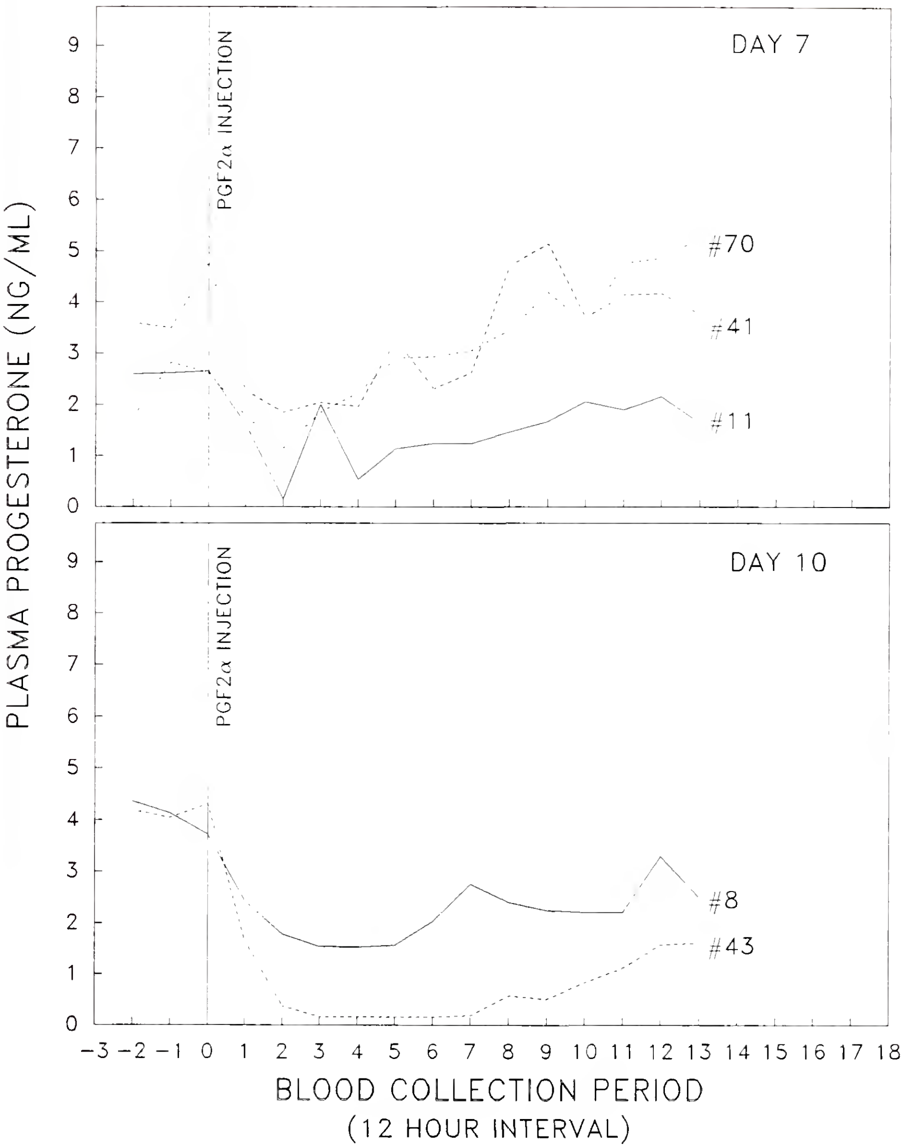

PLASMA PROGESTERONE (NG/ML)
BLOOD COLLECTION PERIOD
(12 HOUR INTERVAL)
NONRESPONDERS
RESPONDERS
PGF2α INJECTION

FIGURE 11. PLASMA P4 CONCENTRATIONS FROM 1 D BEFORE PGF2α FOR ALL NONRESPONDING HEIFERS AFTER PGF2α INJECTION ON D 7 OR D 10 OF THE ESTROUS CYCLE (TRIAL 2).



DAY 7
PGF2α INJECTION
#70
#41
#11
DAY 10
PGF2α INJECTION
#8
#43
PLASMA PROGESTERONE (NG/ML)
0
1
2
3
4
5
6
7
8
9
-3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
BLOOD COLLECTION PERIOD
(12 HOUR INTERVAL)

These data indicate a definite effect of PGF2α on CL function in heifers that either expressed estrus or did not, but the effect was apparently insufficient to cause complete lysis of the CL in "nonresponders." Estrous cycle length in the nonresponders was 20.4 d (normal cycle length). A similar transient decline in P4 concentrations after PGF2α injection has been reported by others (Chenault et al., 1976; Stellflug et al., 1977; Renegar et al., 1978; Refsal and Seguin, 1980; Bosu et al., 1981; Hixon et al., 1983; Maffeo et al., 1983; Stevenson et al., 1987). As with the interval from PGF2α injection to estrus, this temporary decline in plasma P4 may be influenced by the follicular population on the ovary at the time of injection and the ability of the follicles to synthesize and release estradiol. Injection of PGF2α (Chenault et al., 1976) or the analog, cloprostenol, (Schallenberger et al., 1984; Harrison et al., 1985) in cattle produced a rapid elevation in plasma estradiol. Stellflug et al. (1977) also reported a rapid increase in serum estradiol after a luteolytic dose of PGF2α but indicated there was no rise in estradiol concentrations of the PGF2α treated animals that failed to show estrus. These heifers did experience a transitory decrease in P4 concentrations.

Hixon and Hansel (1974) suggested that luteolysis is initiated by PGF2α and a surge of estrogen completes the process. Manns et al. (1975) proposed the exogenous PGF2α may cause a release of endogenous PGF2α which would reinforce the final demise of the CL. The release of endogenous PGF2α, however, is not essential for luteal regression as administration of PGF2α injections produced luteolysis in hysterectomized heifers (LaVoie et al., 1975; Stellflug et al., 1977). Perhaps the presence of a source for estradiol (the follicles) or endogenous PGF2α (the endometrium) plays a supportive role in the induction of luteolysis by exogenous

PGF2α. Animals early in the estrous cycle that fail to express estrus after treatment with PGF2α injection may have insufficiently developed follicles or inadequate levels of endometrial PGF2α to support the initiated luteolysis, so the CL recovers.

Another possible reason for the inablility of a PGF2α injection to induce total luteal regression in all animals might be an inadequate number and(or) affinity of PGF2α receptors present on the CL at the time of treatment. The number of PGF2α receptors on the bovine CL progressively increases from d 3 of the estrous cycle until it peaks on d 20, a time when the CL is actively regressing. Although there are a relatively large number of receptors present on d 13, the affinity of these receptors for PGF2α was 203 times lower than at d 20 (Rao et al., 1979). The sensitivity of the CL to PGF2α during the estrous cycle may be controlled by change in receptor affinity. Heifers in this study that failed to expess estrus after injection on d 7 or d 10 of the estrous cycle may have had inadequate numbers of PGF2α receptors on the CL or the receptors had such a low affinity that the PGF2α failed to precipitate complete luteolysis.

One very simple explanation for the low response rate in this study may be that the PGF2α dose given was too low to effect luteolysis in all heifers. This seems unlikely as other researchers have compared PGF2α dose levels that were higher than the 25 mg dose used in these studies without increasing response rate (Roche et al., 1974 - 20 or 30 mg; Hafs et al., 1975 - 20, 30, 40 or 60 mg; Lauderdale et al., 1981 - 0, 5, 15, 25 or 35 mg) and Ansotegui et al. (1983) reported there was no difference in estrous response rate to 12.5 mg vs a 25 mg injection of PGF2α. Due to the low response rates on d 7 and d 10 in trials 1 and 2 in this study a third trial was

conducted to determine if two injections of PGF2α given 24 h apart would induce estrus more effectively than a single injection.

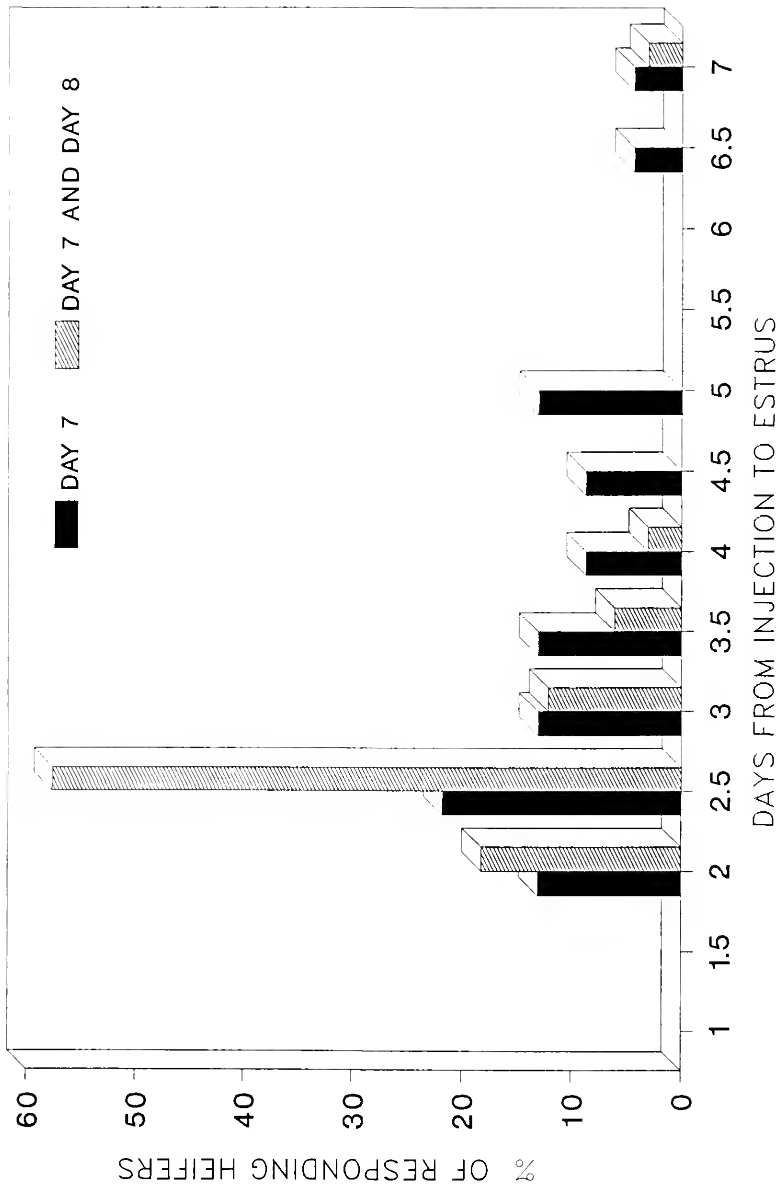
Trial 3

Two injections of PGF2α (25 mg each) administered at a 24 h interval, with the first on d 7 and the second on d 8 of the estrous cycle induced estrus more effectively than a single injection (97% vs 72%, P<.02; table 5). The use of two injections of PGF2α may increase response rate by mimicing the PGF2α release that occurs just prior to natural luteolysis. There are low concentrations of PGF2α in the uterine vein on d 1 to 14 of the estrous cycle with higher concentrations on d 15 to estrus in heifers (Shemesh and Hansel, 1975a). Peaks of PGFM (PGF2α metabolite) were measured in the cow 2 to 3 d prior to estrus (Peterson et al., 1975). High concentrations of PGF2α were released as rapid pulses during d 19 and d 20 of the estrous cycle (Kindahl et al., 1976a). These coincided with a decrease in P4 concentrations (Kindahl et al., 1976b). Thus, the second injection of PGF2α at a time when the P4 is already depressed is sufficient to complete luteolysis in animals that would otherwise have a recovery in plasma P4.

Heifers in this study were AI 12 h after the last PGF2α induced estrus (AM/PM rule) following treatment in the second phase of the study. Overall pregnancy rate was 74% with a first service pregnancy rate of 33 to 38%. There was no difference due to treatment.

Heifers treated with two injections at a 24 h interval were more tightly synchronized than heifers given a single injection (P<.06), with 94% of the double injection heifers expressing estrus in a 36 h period from 2.0 to 3.5 d after the first injection (figure 12). The interval from injection to estrus was shorter in heifers given

FIGURE 12. PERCENT OF RESPONDING HEIFERS EXHIBITING ESTRUS ON SPECIFIC DAYS AFTER $PGF_{2\alpha}$ INJECTION ON D 7 OR D 7 AND D 8 OF THE ESTROUS CYCLE (DEGREE OF SYNCHRONY).


DAY 7
DAY 7 AND DAY 8
% OF RESPONDING HEIFERS
0
10
20
30
40
50
60
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
DAYS FROM INJECTION TO ESTRUS

two injections of $PGF2\alpha$ than in the heifers given a single injection (2.71 ± .15 vs 3.63 ± .29 d; P<.01; table 5). For practical synchronization, a system using a series of three injections of $PGF2\alpha$ with the second injection given 11 d after the first and the third given 24 h after the second would result in more animals in estrus and a tighter synchrony of estrus that could allow successful timed AI (AI by appointment instead of individual animals bred according to onset of estrus). A fourth trial was conducted as a preliminary test of the proposed new synchronization system.

Trial 4

In trial 4, heifers were treated as a group (regardless of day of cycle) with two injections of $PGF2\alpha$ (25 mg) given 24 h apart. All heifers that received the first injection on d 5 or later expressed estrus within 7 d after the first injection. The overall interval to estrus was 3.0 ± .2 d with a range of 1 to 4 d (table 8). It was decided that the proposed new system of synchronization could prove successful under practical ranch conditions. To this end, Santos (1987) conducted field trials and showed the three injection system increased estrual response by 11% and pregnancy rate by 10%. This increase in pregnancy rate was a reflection of the increased rate of response to $PGF2\alpha$. In addition, use of 12.5 mg of $PGF2\alpha$ at the second and third injections was just as effective as the 25 mg dose in this protocol.

During trials 2, 3 and 4 records were kept of the time of day estrus was first observed and circumstances of estrual behavior determination (stood to mounted by bull, by other heifers, or no longer standing to be mounted but previously marked by bull). Summary of these data is shown in table 9. Treatment with $PGF2\alpha$ did not appear to affect the time of day at which estrus was first noticed. In general, an equal number of heifers were seen exhibiting estrus in the morning (AM) as were seen in the

evening (PM). This is in agreement with data reported by others (Galina et al., 1982; Jöchle et al., 1982). Estrus determination was based on seeing the heifers stand to the bull 55 to 58% of the time, stand to other heifers 9 to 23% of the time, or by observing only the marks left by the bull equipped with a chinball marker 22 to 33% of the time.

TABLE 8. ESTRUS RESPONSE AND INTERVAL FROM INJECTION TO ESTRUS OF BRAHMAN HEIFERS TREATED WITH TWO INJECTIONS OF PGF2α GIVEN 24 HOURS APART WITH THE FIRST ON RANDOM DAYS OF THE CYCLE

Days of cycle at PGF2α injection	n	No. in estrus after injections	Days from first injection to estrus
0 and 1	1	0	21.00
1 and 2	1	0	16.00
3 and 4	1	0	18.00
5 and 6	2	2	4.25
7 and 8	2	2	2.50
8 and 9	2	2	3.00
9 and 10	1	1	4.00
10 and 11	1	1	3.00
11 and 12	3	3	3.50
13 and 14	3	3	3.00
14 and 15	1	1	4.00
15 and 16	1	1	3.00
16 and 17	2	2	2.00
17 and 18	2	2	3.25

Overall interval from 1st injection to estrus for responding heifers (mean ± SE) = 3.0 ± .2 days

Interval Range = 1 to 4 days

TABLE 9. CIRCUMSTANCE AND TIME OF DAY (AM OR PM) OF DETECTION OF ESTRUS BEFORE AND AFTER PGF2α TREATMENT (TRIALS 2, 3 AND 4)

	Time of day		Circumstance		
Trial	AM	PM	Stood to bull	Stood to heifer	Marked only
2					
Pre-PGF2α	24	21	32	3	10
Post-PGF2α	14	19	13	11	9
Total	38 (49%)	40 (51%)	45 (58%)	14 (18%)	19 (24%)
3					
Pre-PGF2α	20	11	17	4	10
Post-PGF2α	23	39	34	18	10
Total	43 (46%)	50 (54%)	51 (55%)	22 (23%)	20 (22%)
4					
Pre-PGF2α	11	8	11	3	5
Post-PGF2α	18	6	14	1	9
Total	29 (67%)	14 (33%)	25 (58%)	4 (9%)	14 (33%)

SUMMARY

Heifers injected early in the estrous cycle had lower response rates to a single injection of PGF2α than heifers injected late in the cycle. Use of natural PGF2α did not not adversely affect plasma P4 concentrations in heifers that responded to treatment. This would indicate PGF2α induced a normal functioning CL. All heifers that were injected with PGF2α during trial 2 had plasma concentrations of P4 that declined but heifers that failed to express estrus had concentrations that began to increase by 48 h after treatment and reached a level three times as high as responders by 6 d after injection. A series of two injections of PGF2α given with the first on d 7 and the second on d 8 of the estrous cycle increased rate of response, decreased interval to estrus, and increased degree of synchrony when compared to a single injection on d 7. A series of two injections of PGF2α (given 24 h apart) on random days of the estrous cycle induced estrus in all heifers when the first injection was given on d 5 or later of the cycle. The results of this study led to the proposal of a modified synchronization system using a series of three injections. Subsequent testing of this protocol in field trials proved its success. Use of this new system could improve the AI programs of cattle producers by increasing rates of response and pregnancy. The fact that synchrony was tighter when a series of two injections of PGF2α was used (trial 3) may indicate the possibility of using this new system of synchronization to allow successful program of AI by appointment.

APPENDIX A - RADIOIMMUNOASSAY

RADIOIMMUNOASSAY FOR PROGESTERONE

Reagents

1. Phosphate buffered saline (PBS) - 0.1 M.

    16.35 g $Na_2HPO_4 \cdot 7H_2O$ (MW 268.07)

    5.40 g $Na_2HPO_4 \cdot H_2O$ (MW 137.99)

    9.00 g NaCl (MW 58.44)

    1.00 g NaN_3 (MW 65.01)

    - Place in 1 liter volumetric flask and dissolve in ≈ 900 ml deionized H_2O. Adjust to pH 7.5 with concentrated NaOH and bring volume to 1 liter with deinonized H_2O.

2. Phosphate buffered saline with gelatin (PBSG) - 0.1 M

    - Dissolve 1 g gelatin in 1 liter of 0.1 M PBS. May be stored at 4°C for up to 1 week.

3. Dextran coated charcoal (DCC) solution

    0.625 g Norit A charcoal (Matheson, Coleman and Bell Mfg. Chemicals, Norwood, OH)

    0.0625 g dextran - grade C (Becton Dickerson, Rutherford, NJ)

    - Add to 100 ml PBSG. May be stored at 4°C for up to 2 weeks.

4. P4 stock solution (10 μg/ml)

    - 500 μg P4 in 50 ml distilled benzene. Store in sealed flask at 4°C until used to make "A" standard.

5. $[^{1,2-3}H]$-P4 stock solution

- Add 5 ml distilled benzene to vial of tritiated P4 (specific activity = 53.4 Ci/mmol) and vortex. Determine cpm/μl and store in sealed vial at 4°C.

6. "A" standard P4 (1000 pg P4/200 μl PBSG)

- Pipet 50 μl of P4 stock solution into a flask and evaporate benzene under nitrogen gas. Add 100 ml PBSG, cover and stir on a warm stirplate overnight. Aliquot 2 ml of "A" standard into 12 X 75 mm borosilicate culture tubes, cap tightly, and store at -20°C.

7. Triton X cocktail

- Scintillation cocktail

  0.1 g BisMSB

  3.89 g PPO

  1 liter toluene

- Triton X cocktail

  Triton X 100 (Research Products International Corp., Grove Hill, IL)

  scintillation cocktail

- Combine 1 part Triton X 100 and 3 parts scintillation cocktail and mix completely.

8. Hexane:benzene extraction solvent

- Distill hexane (at 80°C) and benzene (at 69°C) immediatedly prior to each assay. Mix freshly distilled hexane and benzene at a 1:2 ratio for extraction.

<u>Extraction of Plasma Samples - Ether as solvent</u>

1. Dry down an aliquot of $[^{1,2-3}H]$-P4 stock solution sufficient to prepare a

[$^{1,2-3}$H]-P4-PBSG solution with approximately 1500 cpm/100 μl (recovery P4).

2. Pipet 200 μl of plasma sample into a 20 X 150 mm screw top glass tube.
3. Pipet triplicates of 200 μl of plasma from a cow at diestrus (standard plasma) into 20 X 150 mm glass tubes to assay for determination of intra- and interassay coefficient of variation.
4. Add 100 μl of recovery P4 to all tubes and vortex well.
5. Add 5 ml ether to each tube containing 200 μl plasma, cap and vortex.
6. Allow to stand for 5 min, remove cap and freeze plasma by briefly plunging lower half of tube into liquid nitrogen.
7. Decant supernatant (be sure pellet remains frozen) into 16 X 100 mm borosilicate tubes.
8. Evaporate ether in the 16 X 100 mm tubes with nitrogen gas under a fume hood.
9. Add 2 ml PBSG to each sample tube and vortex very well to resolubilize the dried sample.
10. Aliquot 200 μl of each extracted sample and tripilicates of 100 μl of recovery P4 into scintillation vials. Add 3 ml Triton X cocktail and count each vial for 10 min in a liquid scintillation counter to determine % recovery for the assay.

<u>Extraction of Plasma Samples - Hexane:Benzene as Solvent</u>

- Proceed as above but add 2 ml of hexane:benzene extraction solvent to the plasma samples instead of 5 ml of ether. Vortex well and plunge extracted plasma in ethanol and dry ice until pellet is frozen. Alternatively, extracted plasma may be frozen by placing tubes in a -20°C freezer. Be sure pellet remains frozen until supernatant is poured off.

Radioimmunoassay for P4

1. Dry down sufficient [$^{1,2-3}$H]-P4 stock solution under nitrogen gas to produce a solution with ≈ 14,000 to 16,000 cpm/100 μl PBSG. Add appropriate quantity of PBSG and vortex well (total count solution, TCS).
2. Serial dilute the "A" standard to prepare a set of standard curves in duplicate. Standards for the assay should be 1000, 500, 250, 125, 62.5, 31.2, and 15.6 pg/200 μl PBSG.
3. Prepare antibody solution by diluting antiserum to achieve a total binding of 31 to 45% (P4-Ab).
4. Aliquot volumes of standards, samples, total count solution, P4-Ab, and PBSG into 10 X 75 mm borosilicate tubes as indicated in appendix table 1. Add everything but TCS and incubate for 30 min at room temperature. After 30 min add TCS, vortex, and incubate for 6 to 18 h at 4°C.
5. Add 200 μl of DCC (charcoal solution) to all tubes except the total count tubes and vortex briefly. Incubate for 15 min at 4°C and then centrifuge at 3500 rpm for 15 min. While adding the DCC to the assay tubes be sure the charcoal remains in solution by stirring gently on a stirplate.
6. Decant supernatant (be very careful to avoid disturbing pellet) in liquid scintillation vials and add 3 ml of Triton X cocktail.
7. Cap and count each vial for 5 min.

Validation of P4 Assays

- Validation of P4 assays for trials 1 and 2 were conducted by adding to five replicates 1000, 500, 250, 125, 61.5, and 31.3 pg P4/ml plasma from an ovariectomized cow. Plasma samples were then extracted and

radioimmunoassayed for P4 as described above. In trial 1 the antibody used was supplied by Dr. L. Fleeger of Texas A & M University, College Station and in trial 2 the antibody was supplied by Dr. J. Troconiz and Dr. M. de Manzo of the Universidad Central Venezuela, Marcay. Concentrations of P4 added to the plasma and measured by assay are shown in appendix tables 2 and 3.

APPENDIX TABLE 1. ALIQUOT VOLUMES FOR P4 ASSAY (μl)

Tube	Standard or sample	PBSG	P4-Ab	TCS
Standards	200	300	100	100
Total count	---	800	---	100
Total binding	---	500	100	100
Non-specific binding	---	600	---	100
Samples	200	300	100	100

APPENDIX TABLE 2. VALIDATION FOR P4 ASSAY
- DR. L. FLEEGER ANTIBODY

P4 added to plasma (pg/ml)	no. of replicates	P4 measured (pg/ml) (mean ± SE)
1000	5	949.2 ± 21.8
500	5	421.6 ± 36.5
250	5	247.4 ± 14.3
125	5	129.2 ± 3.2
62.5	5	42.8 ± 5.2
31.3	5	14.4 ± 3.3

APPENDIX TABLE 3. VALIDATION FOR P4 ASSAY - VENEZUELAN ANTIBODY

P4 added to plasma (pg/ml)	no. of replicates	P4 measured (pg/ml) (mean ± SE)
1000	5	950.8 ± 22.9
500	5	405.3 ± 17.6
250	5	214.8 ± 18.5
125	5	121.3 ± 5.3
62.5	5	73.4 ± 8.7
31.3	5	50.5 ± 7.8

APPENDIX B - RAW DATA

APPENDIX TABLE 4. WEIGHTS OF HEIFERS FROM BIRTH THROUGH TRIAL 1 (LB)

ANIM	SIRE	BIRTHDATE	BIRTHWT	WEANWT 9/5/80	12/20/80	2/19/81	6/19/81	8/20/81	11/12/81	12/10/81	1/7/82	2/4/82	3/4/82	4/1/82	5/13/82	6/24/82
1	7715	12-23-79	67	509	600	658	788	848	905	942	947	928	943	976	1017	1063
2	7715	12-27-79	60	481	573	634	777	845	861	905	943	955	934	956	1005	1029
4	7715	1-07-80	55	492	594	695	814	863	885	925	957	980	955	964	1011	1078
7	335	1-07-80	70	467	547	610	747	812	875	900	924	914	942	945	982	1014
8	764	1-09-80	80	493	591	675	846	897	930	982	1010	1049	1029	1070	1109	1176
11	7715	1-12-80	69	456	527	588	744	836	835	857	888	911	876	912	979	1037
16	723	1-18-80	60	421	498	567	690	723	738	767	806	832	810	832	880	924
18	764	1-19-80	81	439	534	630	800	880	911	942	980	991	956	1000	1066	1136
19	764	1-19-80	70	496	602	665	810	900	935	936	987	960	974	1002	1074	1153
23	598	1-20-80	64	429	516	580	701	750	802	845	852	855	859	863	882	952
25	406	1-21-80	80	541	627	740	904	972	985	1016	1083	1061	1051	1106	1177	1244
28	723	1-22-80	60	550	651	755	922	980	954	1034	1072	1083	1058	1086	1142	1204
35	764	1-26-80	76	532	639	725	874	950	955	995	1046	1053	1045	1054	1109	1173
36	418	1-26-80	69	427	511	590	766	842	880	910	964	944	907	935	968	976
37	7715	1-26-80	71	469	549	622	773	833	865	864	900	928	908	949	977	1030
39	7715	1-29-80	75	410	516	600	756	875	870	915	915	927	892	952	998	1081
45	335	2-04-80	60	394	493	567	743	810	811	832	861	846	856	881	930	988
48	723	2-05-80	75	452	559	657	799	878	920	930	980	995	984	1014	1080	1147
50	406	2-06-80	78	411	513	585	723	815	820	877	906	907	890	919	950	1008
51	418	2-07-80	73	487	598	669	817	925	930	938	954	985	943	989	1003	1058
54	418	2-09-80	85	490	570	649	799	923	910	945	973	1005	960	997	1032	1081
59	418	2-18-80	76	474	580	658	810	867	910	931	972	984	981	1000	1044	1107
61	764	2-20-80	70	461	543	612	779	822	860	887	930	959	952	1000	1026	1095
64	406	2-24-80	70	390	473	580	649	725	745	754	786	799	785	824	848	904
72	723	3-05-80	65	365	451	530	669	733	785	785	808	834	854	847	879	927
73	335	3-05-80	65	388	483	555	698	748	785	823	863	861	882	884	929	992
74	723	3-06-80	80	414	532	605	740	815	800	853	883	897	881	930	977	1030
76	406	3-06-80	76	394	508	597	747	830	831	873	897	911	924	945	1000	1066
77	7715	3-07-80	73	424	485	545	711	790	800	825	879	872	879	936	990	1038
79	418	3-14-80	70	357	416	525	701	815	825	865	888	894	895	924	961	1021
82	335	3-18-80	82	359	442	512	664	735	740	776	818	841	872	871	931	958
83	598	3-19-80	70	372	442	505	654	768	765	807	852	821	823	844	883	942

APPENDIX TABLE 5. WEIGHTS OF HEIFERS FROM BIRTH THROUGH TRIAL 2 (LB)

ANIM	SIRE	BIRTHDATE	BIRTHWT	6/11/81	11/18/81	1/13/82	3/10/82	5/6/82	6/25/82	9/13/82	11/22/82	12/20/82	2/14/83	4/21/83	9/7/83
104	598	11-12-80	65	510	578	628	660	734	800	830	869	917	944	957	1059
105	418	11-13-80	75	715	760	841	868	924	1006	1020	1091	1115	1157	1203	1234
107	598	12-17-80	70	597	621	715	760	835	867	902	977	1020	1035	1076	1188
108	764	12-18-80	74	531	609	675	743	814	890	931	998	1041	1105	1117	1195
111	598	1-07-81	60	568	620	698	771	856	936	985	1037	1083	1124	1133	1290
112	335	1-08-81	62	480	523	577	615	680	733	789	830	863	862	925	1055
113	7715	1-10-81	65	518	530	618	673	774	854	901	969	1008	1024	1084	1175
116	335	1-14-81	65	510	558	605	638	690	755	800	873	875	903	921	1055
120	764	1-17-81	62	451	501	570	597	644	712	757	822	856	884	890	1020
122	418	1-19-81	75	500	544	618	629	687	744	784	834	861	900	910	1040
124	764	1-22-81	57	425	510	565	568	632	685	748	765	780	805	764	890
125	335	1-23-81	73	527	551	610	685	763	851	861	935	970	1008	1059	1191
126	764	1-25-81	90	422	442	525	565	641	721	775	852	889	959	983	1137
128	7715	1-26-81	70	472	532	600	657	753	836	876	922	960	1017	1050	1172
130	764	1-27-81	60	520	571	630	669	731	830	852	912	930	944	979	1123
137	598	1-31-81	66	546	591	650	702	754	839	877	961	1000	1083	1105	1245
138	7715	1-31-81	95	521	565	660	718	787	896	971	1049	1099	1147	1195	1387
141	7715	2-01-81	70	563	498	557	564	626	708	758	829	845	880	865	1060
143	764	2-03-81	80	477	489	555	591	662	731	769	817	822	875	907	1035
146	418	2-08-81	60	520	556	625	672	773	840	866	959	985	1056	1110	1192
147	7715	2-09-81	70	427	540	600	640	691	755	800	871	900	899	965	1085
148	7715	2-09-81	72	446	510	613	676	747	826	875	934	961	991	1055	1163
151	418	2-10-81	85	506	587	628	697	779	831	881	962	984	1061	1082	1262
155	418	2-16-81	84	509	567	638	681	767	866	887	974	990	1055	1107	1212
158	328	2-20-81	85	438	484	565	610	689	770	839	908	936	971	1035	1168
162	335	2-26-81	65	404	435	504	546	621	696	750	807	827	857	890	1015
164	335	2-28-81	62	425	476	535	577	631	697	717	826	844	880	902	1071
166	337	3-02-81	80	416	467	535	541	631	685	745	788	816	845	865	1041
169	598	3-05-81	80	373	453	520	545	642	757	831	873	945	973	1015	1190
170	418	3-08-81	75	451	457	520	556	661	757	821	899	912	948	980	1157

APPENDIX TABLE 6. PLASMA P4 CONCENTRATIONS BEFORE TREATMENT (PG/ML) - TRIAL 1

ANIM	DAY OF CYCLE												
	2	3	4	5	6	7	8	9	10	11	12	13	14
TREATMENT 1A - ONLY INJECTION ON DAY 7													
28	1210	1420	4030	4220	4660	6370	8520	6790	10260	10560	12840	11480	11910
50	630	690	1210	1850	3820	4590	4980	4290	7180	6740	8160	7130	9270
51	630	630	1190	2460	3310	3850	5260	5580	5850	8100	5010	8030	7520
59	630	1580	2040	3160	5410	4090	4040	7090	7420	8060	9820	8450	10740
72	630	630	630	1560	2650	2900	3100	4400	4870	5200	6430	4700	5780
74	770	950	1240	2030	2670	4160	6280	5500	5600	6820	6940	8390	5800
TREATMENT 2A - FIRST INJECTION ON DAY 7, SECOND 11 DAYS LATER													
1	630	630	1150	2120	3720	4160	3940	4830	6320	6440	5940	7430	7800
23	990	1470	3100	4220	3740	5110	7090	8760	7940	9380	8240	7250	11140
35	630	630	2940	3270	4730	5560	7120	4600	6390	7060	6360	7580	6310
45	630	900	630	1830	2450	2150	2250	2200	2480	2810	1700	2230	2770
61	650	630	2460	3400	5180	5700	6550	7060	7380	7420	8620	10290	9840
83	630	890	1530	3500	5850	8260	8460	7110	7510	9580	9700	7580	7860
TREATMENT 1B - ONLY INJECTION ON DAY 14													
2	630	630	630	1000	2140	3280	3660	4700	5720	6000	6460	6820	6340
4	630	630	630	4340	2690	3290	3730	4030	3770	5020	6160	5210	4990
8	630	1010	1090	2640	4080	5360	5040	6630	4020	5900	6390	5110	5290
11	630	690	2020	3090	4640	3910	6780	5350	6920	6100	7510	6390	5980
25	1170	820	1920	2610	4950	5100	6270	5180	6970	7700	6680	8160	7370
48	1570	1980	2340	4340	3480	5210	5390	6790	7740	10120	7820	9590	10000
TREATMENT 2B - FIRST INJECTION ON DAY 14, SECOND 11 DAYS LATER													
16	630	3240	1970	2520	3740	2860	4300	5790	4770	*	3520	7150	7000
18	630	630	960	1570	2440	2890	3210	3030	3730	4100	5900	4560	6150
19	630	2090	1340	1700	3370	3710	5130	7030	6060	6380	4570	*	3800
36	630	770	1870	2470	3500	3610	4360	5740	8030	5930	5240	8180	8380
37	630	630	1500	2430	3240	3450	4750	5370	5610	5490	4820	5810	4920
64	630	800	1600	2840	3580	4670	4150	4000	5220	5260	4320	5150	5130
TREATMENT C - NO TREATMENT													
7	630	1120	1150	3050	2980	2560	3740	5280	5570	5230	5110	7010	4330
39	710	840	630	900	2270	3410	4210	5410	4210	5290	4730	5410	4470
54	630	630	630	1040	2290	3170	3120	4670	6630	5260	3610	6060	8030
73	630	630	630	1340	1610	2620	3360	4690	5130	4670	6740	5140	6850
76	640	1870	2080	4170	2180	4080	2650	5560	6430	6130	9000	6280	6170
77	740	1180	740	1420	2740	2860	2680	4500	4580	4950	4570	6960	6170
79	3300	3110	4770	7950	7060	8300	8070	8550	8530	10450	9780	8450	7440
82	3410	4710	2290	2180	*	3410	3060	4380	*	4310	3900	2540	2610

* = MISSING DATA POINT

APPENDIX TABLE 7. PLASMA P4 CONCENTRATIONS AFTER TREATMENT (PG/ML) - TRIAL 1

ANIM	RESP	DAY OF CYCLE												
		2	3	4	5	6	7	8	9	10	11	12	13	14
TREATMENT 1A - ONLY INJECTION ON DAY 7														
28	Y	1100	1380	2860	4760	5140	5080	10260	8040	8080	11660	13040	11230	12010
50	N	*	6250	5080	4850	5690	6080	6750	7400	9120	9010	8860	8640	2480
51	Y	660	3380	2080	2910	3650	4360	5770	6520	5710	8110	7050	8370	7210
59	Y	770	630	770	1480	2650	3210	3650	5330	6460	5040	5940	5970	6340
72	N	4820	3570	1600	630	630	630	630	630	630	1720	1730	2560	4760
74	Y	780	640	1290	2140	3510	3940	6660	5590	7080	9490	10360	8360	6400
TREATMENT 2A - FIRST INJECTION ON DAY 7, SECOND 11 DAYS LATER														
1	N	*	*	*	5400	6650	4720	6590	6200	6150	6880	7150	6990	1350
23	Y	2220	990	1970	2410	4120	4320	4510	6080	6610	6100	6590	6390	9160
35	Y	630	630	1750	1170	2870	3470	2970	4420	4370	5130	6130	5630	5200
45	Y	1130	1020	1350	2450	3090	3120	3900	4330	3620	6010	3740	4600	4240
61	Y	6600	7450	7650	7130	7320	9100	9690	8190	7060	8590	8650	4640	950
83	N	4780	4030	1130	630	630	630	1100	2190	3400	4930	6060	6830	8550
TREATMENT 1B - ONLY INJECTION ON DAY 14														
2	Y	630	990	1010	2680	3670	3600	3410	6300	6250	4020	4940	5020	7480
4	Y	630	630	1500	1950	2110	2710	3310	5670	4910	4810	4170	7250	3610
8	Y	890	1500	2980	3220	3450	4640	4370	6360	5850	5640	7670	7580	8150
11	Y	630	630	1430	2150	2980	4340	4040	5280	5490	5670	5920	5170	5770
25	Y	1110	1650	1850	3660	4360	6510	4680	7490	8880	5650	7090	5820	7120
48	Y	920	920	1580	2300	2680	4200	4880	6420	4220	6240	7980	6220	7990
TREATMENT 2B - FIRST INJECTION ON DAY 14, SECOND 11 DAYS LATER														
16	N	2530	4900	5040	630	630	630	630	630	630	630	1500	2310	2770
18	Y	630	630	1680	2380	3000	4720	3330	3810	4720	3880	3420	3100	3620
19	Y	630	630	1270	1540	2850	4080	3220	5540	5390	6820	6530	6730	5210
36	Y	630	630	630	2180	2720	4270	3670	3930	5730	5190	6380	5740	6160
37	N	4290	1970	2650	630	630	630	630	630	630	630	1780	2430	2220
64	Y	630	630	1290	1690	2830	3020	4390	4550	3860	5280	4370	4110	4900
TREATMENT C - NO TREATMENT														
7	-	630	1610	1690	3010	2750	2920	3730	2980	4520	3580	3290	4530	4300
39	-	630	630	630	1860	2860	3640	4260	3950	5900	7870	5550	6440	5230
54	-	630	630	1000	1710	4010	4690	4060	5770	6030	5500	7720	5580	8710
73	-	630	630	1050	1610	2610	2830	3980	4690	5400	5960	5160	4830	5650
76	-	630	630	630	1590	2160	3530	4530	4630	5700	4280	6390	5810	4960
77	-	630	770	2010	3340	3360	3400	4550	4900	6220	6090	7670	7860	7020
79	-	2540	4220	3400	2290	4730	4300	8100	6170	8320	7550	8290	4370	5180
82	-	3050	2310	2100	1770	1740	2070	2130	2770	2720	3830	2580	2460	2150

* = MISSING DATA POINT Y = ESTRUS N = NO ESTRUS

APPENDIX TABLE 8. PLASMA P4 CONCENTRATIONS AFTER TREATMENT (PG/ML) - TRIAL 2

ANIM	RESP	COLLECTION PERIOD -2	-1	INJ	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
TREATMENT 1 - DAY 7																					
11	N	2591	2612	2648	1615	156	1998	534	1140	1241	1246	1457	1671	2057	1897	2148	1640	*	*	*	*
22	Y	2305	2706	2638	1035	1013	672	567	285	156	156	755	1040	1249	1743	*	*	*	*	*	*
30	Y	2938	3813	3461	1232	440	227	1374	497	894	839	770	765	619	358	661	1040	1271	1728	*	*
41	N	3593	3487	4771	2266	1849	2040	1957	3264	2312	2624	4706	5140	3684	4133	4156	3747	*	*	*	*
51	Y	3380	4130	4805	2356	1203	1151	976	1047	156	710	1249	913	1054	1459	*	*	*	*	*	*
70	N	1751	2818	2612	1769	1151	1847	2226	2905	2928	3049	3438	4182	3555	4746	4849	5251	*	*	*	*
TREATMENT 2 - DAY 10																					
8	N	4365	4130	3710	2431	1780	1541	1527	1569	2033	2750	2402	2244	2202	2204	3287	2498	*	*	*	*
26	Y	4908	5389	5978	2065	946	665	865	593	572	345	543	684	156	156	914	1130	1701	2236	*	*
37	Y	3556	3377	3659	1875	1098	572	593	662	269	760	680	584	584	779	779	593	738	779	812	1319
43	N	4189	4042	4303	1660	373	156	156	156	156	189	576	512	828	1112	1571	1608	*	*	*	*
47	Y	2983	2010	3764	1842	1150	682	403	468	156	156	652	156	156	283	*	*	*	*	*	*
62	Y	3823	5133	3876	2076	1486	1437	313	727	1146	364	430	1087	912	1660	1343	1029	1026	945	2152	1226
TREATMENT 3 - DAY 14																					
12	Y	7844	4118	7834	4991	3006	2531	1071	157	1187	1999	1327	1945	884	2104	*	*	*	*	*	*
16	Y	7581	5760	9392	3509	2508	890	190	415	1363	1137	157	157	157	157	236	157	674	1363	*	*
28	Y	6030	7138	5617	2074	1794	1300	1650	1384	157	1269	914	1446	684	1816	2555	2608	*	*	*	*
58	Y	5660	6032	4675	3605	1561	901	313	725	568	633	313	624	972	313	1030	711	1428	1762	*	*
64	Y	4502	8460	5360	3038	1794	1145	1472	1923	922	869	759	809	313	681	1110	1252	*	*	*	*
66	Y	4712	7130	5287	2190	1110	413	937	1088	313	259	774	259	831	1663	*	*	*	*	*	*

* = MISSING DATA POINT

APPENDIX TABLE 8. CONTINUED

ANIM	RESP	COLLECTION PERIOD																			
		-2	-1	INJ	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
TREATMENT 4 - DAY 18																					
5	Y	3718	4176	6048	2268	1918	1448	156	327	876	747	1169	1347	378	784	907	876	*	*	*	*
7	Y	4990	3784	3868	1490	1899	1002	1061	581	156	982	1046	1309	1086	1241	997	*	1217	1149	*	*
13	Y	3975	2660	1405	1491	1231	494	1431	1177	611	157	157	157	387	811	*	*	*	*	*	*
25	Y	6035	5809	1608	1652	1800	1484	1676	1307	260	1114	1134	1515	1396	1534	156	190	*	*	*	*
48	Y	5635	6333	4436	1849	875	294	759	156	158	478	278	468	738	585	832	717	*	*	*	*
69	Y	4073	4621	1837	690	344	289	653	376	182	156	641	850	1602	2290	962	1527	*	*	*	*
TREATMENT 5 - CONTROL																					
4	-	6744	8498	5626	2561	1634	1731	1392	156	310	1078	1252	1290	639	816	1848	1623	*	*	*	*
20	-	7824	7893	11233	10993	7464	6904	6079	3014	1395	1005	721	797	310	1083	854	803	661	1131	1346	1767
24	-	1257	1167	915	861	970	854	1135	757	1016	910	1099	*	*	*	*	*	*	*	*	*
38	-	7853	6515	5162	2393	915	862	1037	1150	574	653	138	1085	156	1042	1248	1471	2203	2372	2944	3161
46	-	4259	1648	1638	825	1000	859	897	462	995	696	829	683	1275	1305	1562	*	*	*	*	*
55	-	3348	3522	4162	5740	5035	4845	5304	4506	3946	1346	1168	714	912	780	761	156	*	*	*	*

* = MISSING DATA POINT

APPENDIX TABLE 9. ESTRUAL RESPONSE TO PGF2α - TRIAL 3

ANIM	RESP	TIME	ESTRUS TYPE	INTERVAL TO ESTRUS (DAYS)	ANIM	RESP	TIME	ESTRUS TYPE	INTERVAL TO ESTRUS (DAYS)
PHASE 1					PHASE 2				
ONE INJECTION - DAY 7					ONE INJECTION - DAY 7				
54	N	PM	+	9.0	76	N	PM	+	10.0
73	Y	AM	o	3.0	82	Y	PM	+	2.5
76	N	PM	*	10.0	105	Y	PM	o	3.5
120	Y	AM	-	3.0	138	Y	PM	+	2.5
146	Y	AM	*	7.0	146	Y	PM	+	3.5
170	N	AM	*	12.0	170	N	AM	*	9.0
208	Y	PM	*	2.5	201	Y	AM	*	4.0
211	Y	PM	*	4.5	205	Y	PM	*	2.5
226	Y	AM	*	2.0	226	Y	AM	*	2.0
234	Y	AM	-	5.0	236	Y	PM	*	6.5
236	Y	PM	*	4.5	241	Y	PM	*	2.5
255	N	AM	*	11.0	265	Y	AM	o	2.0
260	N	PM	+	10.0	269	Y	AM	o	5.0
265	Y	AM	-	5.0	274	Y	PM	+	3.5
274	Y	AM	*	4.0	281	N	PM	+	13.0
281	N	AM	+	13.0	291	Y	AM	o	3.0
TWO INJECTIONS - DAY 7 AND DAY 8					TWO INJECTIONS - DAY 7 AND DAY 8				
28	Y	PM	*	2.5	28	Y	AM	*	2.0
82	Y	PM	*	2.5	54	Y	PM	*	2.5
105	Y	PM	*	3.5	73	Y	PM	*	2.5
138	Y	PM	+	2.5	120	Y	PM	+	2.5
158	Y	PM	+	2.5	158	Y	PM	o	7.0
201	Y	AM	*	2.0	208	Y	AM	o	3.0
205	Y	AM	o	3.0	211	Y	PM	*	2.5
212	Y	AM	*	2.0	212	Y	PM	*	2.5
229	Y	PM	*	2.5	229	Y	PM	*	2.5
240	Y	PM	+	2.5	234	Y	AM	*	3.0
241	Y	AM	o	3.0	240	Y	AM	*	2.0
247	Y	AM	+	2.0	247	Y	PM	o	2.5
249	Y	PM	+	2.5	249	Y	AM	*	2.0
269	Y	PM	o	3.5	255	Y	AM	+	4.0
271	Y	PM	+	2.5	260	Y	PM	+	2.5
273	N	PM	*	15.0	271	Y	PM	*	2.5
291	Y	PM	*	2.5	273	Y	PM	*	2.5

- = MISSING DATA
* = STANDING ESTRUS TO BULL
+ = STANDING ESTRUS TO OTHER FEMALE
o = NO LONGER STANDING BUT PREVIOUSLY MARKED BY BULL

APPENDIX TABLE 10. ESTRUAL RESPONSE TO PGF2α - TRIAL 4

ANIM	DAY OF CYCLE AT INJECTION	RESP	TIME	ESTRUS TYPE	INTERVAL TO ESTRUS (DAYS)
301	5,6	Y	PM	*	2.5
303	7,8	Y	AM	o	3.0
304	13,14	Y	AM	o	3.0
308	9,10	Y	AM	o	4.0
312	11,12	Y	AM	*	3.0
313	3,4	N	AM	*	18.0
314	13,14	Y	PM	*	2.5
316	16,17	Y	AM	o	3.0
321	17,18	Y	AM	*	4.0
322	11,12	Y	PM	*	3.5
325	15,16	Y	AM	o	3.0
328	14,15	Y	AM	*	4.0
329	8,9	Y	AM	*	4.0
330	1,2	N	AM	o	16.0
337	0,1	N	PM	o	21.0
338	5,6	Y	AM	*	2.0
347	8,9	Y	AM	*	2.0
350	11,12	Y	AM	+	4.0
363	7,8	Y	AM	*	2.0
379	17,18	Y	PM	*	2.5
385	13,14	Y	PM	*	3.5
3157	16,0	Y	AM	o	1.0
3174	10,11	Y	AM	*	3.0

* = STANDING ESTRUS TO BULL
+ = STANDING ESTRUS TO OTHER FEMALE
o = NO LONGER STANDING BUT PREVIOUSLY MARKED BY BULL

APPENDIX C - STATISTICS

APPENDIX TABLE 11. MODEL 1 USED TO TEST FOR HETEROGENEITY OF REGRESSION (P4 DATA) - TRIAL 1

Source	df	Sum of squares	Mean square
Treatment	4	163507421.2331	
Response	1	64167613.2321	
Trt*Res	2	197447406.0430	
Animal (Trt*Res)	24	316949370.3437	
Day	1	4734113.0367	
D*D	1	24718237.9838	
D*D*D	1	31170320.7252	
Model	34	1816901646.3142	53438283.7151
Error	377	841453031.3556	2231970.9055

APPENDIX TABLE 12. MODEL 2 USED TO TEST FOR HETEROGENEITY OF REGRESSION (P4 DATA) - DIFFERENCE DUE TO TREATMENT - TRIAL 1

Source	df	Sum of squares	Mean square
Treatment	4	17183341.3171	
Response	1	60962212.5725	
Trt*Res	2	202644859.9881	
Animal (Trt*Res)	24	315483517.6366	
Day*Trt	5	11776557.6809	
D*D*Trt	5	30622266.8205	
D*D*D*Trt	5	37024153.4714	
Model	46	1931542385.9726	41990051.8690
Error	365	726812291.6973	1991266.5526

df	Model sum of squares for error
377	841453031.3557 (Model 1)
-365	-726812291.6973 (Model 2)
12	114640739.6584 ÷ 1991266.5526 (MSE Model 2) = 57.5717

F Value (12,365) = 57.5717 ÷ 12 = 4.80

$P < .01$

APPENDIX TABLE 13. MODEL 3 USED TO TEST FOR HETEROGENEITY OF REGRESSION (P4 DATA) - DIFFERENCE DUE TO RESPONSE - TRIAL 1

Source	df	Sum of squares	Mean square
Treatment	4	181560433.5121	
Response	1	48189200.3925	
Trt*Res	2	223567908.1584	
Animal (Trt*Res)	24	318961609.9116	
Day*Res	2	25046610.2669	
D*D*Res	2	32627153.5177	
D*D*D*Res	2	35361970.8747	
Model	37	2095018670.0307	56622126.2170
Error	374	563336007.6392	1506246.0097

df	Model sum of squares for error
377	841453031.3557 (Model 1)
-374	-563336007.6392 (Model 3)
3	278117023.7165 ÷ 1506246.0097 (MSE Model 3) = 184.6425

F Value (3,374) = 184.6425 ÷ 3 = 61.55

P < .001

APPENDIX TABLE 14. CHI-SQUARE ANALYSIS OF SYNCHRONIZATION RATES TO PGF2α INJECTION ON D 7 OR D 14 (TRIALS 1 AND 2 COMBINED)

Source	df	Chi-square	P<
Intercept	1	1.26	0.2610
Response	1	3.89	0.0487
Year	1	0.03	0.8610
Response*Year	1	0.03	0.8610

APPENDIX TABLE 15. MODEL 1 USED TO TEST FOR HETEROGENEITY OF REGRESSION (P4 DATA) - TRIAL 2

Source	df	Sum of squares	Mean square
Treatment	4	41399163.8942	
Response	1	54173407.4027	
Trt*Res	1	10761103.2678	
Animal (Trt*Res)	23	82178585.1129	
Period	1	5424910.3310	
P*P	1	3606550.6721	
P*P*P	1	2052385.7593	
Model	32	215621588.2864	6738174.6340
Error	334	228755233.0542	684895.9073

APPENDIX TABLE 16. MODEL 2 USED TO TEST FOR HETEROGENEITY OF REGRESSION (P4 DATA) - DIFFERENCE DUE TO TREATMENT - TRIAL 2

Source	df	Sum of squares	Mean square
Treatment	4	2298789.5399	
Response	1	51429919.0722	
Trt*Res	1	9576748.3397	
Animal (Trt*Res)	23	88744086.5450	
Period*Trt	5	5945663.4353	
P*P*Trt	5	4074164.7163	
P*P*P*Trt	5	3183385.4203	
Model	44	273389812.1369	6213404.8213
Error	322	170987009.2037	531015.5565

df	Model sum of squares for error
334	228755233.0542 (Model 1)
-322	-170987009.2037 (Model 2)
12	57768223.8505 ÷ 531015.5565 (MSE Model 2) = 108.78

F Value (12,322) = 108.78 ÷ 12 = 9.06

P < .01

APPENDIX TABLE 17. MODEL 3 USED TO TEST FOR HETEROGENEITY OF REGRESSION (P4 DATA) - DIFFERENCE DUE TO RESPONSE - TRIAL 2

Source	df	Sum of squares	Mean square
Treatment	4	40397838.5023	
Response	1	500008.5407	
Trt*Res	1	11038299.9907	
Animal (Trt*Res)	23	82923679.1519	
Day*Res	2	5621672.8463	
D*D*Res	2	3112160.1874	
D*D*D*Res	2	1483150.6397	
Model	35	252195227.2019	7205577.9201
Error	331	192181594.1387	580609.0457

df	Model sum of squares for error
334	228755233.0542 (Model 1)
-331	-192181594.1387 (Model 3)
3	36573638.9155 ÷ 580609.0457 (MSE Model 3) = 62.99

F Value (3,331) = 62.99 ÷ 3 = 21.00

P < .001

APPENDIX TABLE 18. CHI-SQUARE ANALYSIS OF SYNCHRONIZATION RATES TO TREATMENT WITH EITHER 1 OR A SERIES OF 2 INJECTIONS OF PGF2α WITH THE SECOND INJECTION GIVEN 24 H AFTER THE FIRST - TRIAL 3

Source	df	Chi-square	P<
Intercept	1	18.74	0.0001
Treatment	1	5.82	0.0159

APPENDIX TABLE 19. CHI-SQUARE ANALYSIS OF SYNCHRONIZATION RATES ON THE 7 D FOLLOWING EITHER 1 OR A SERIES OF 2 INJECTIONS OF PGF2α WITH THE SECOND GIVEN 24 H AFTER THE FIRST (DEGREE OF SYNCHRONY) - TRIAL 3

Statistic	df	Value	P<
Chi-square	8	14.52	0.061
Likelihood Chi-square	8	16.93	0.031
Mantel-Haenszel Chi-square	1	7.90	0.005

APPENDIX TABLE 20. T-TEST FOR INTERVAL FROM PGF2α INJECTION (LAST OR ONLY) TO ESTRUS - TRIAL 3

Treatment	n	Mean ± SE
PGF2α - d 7	23	3.63 ± .29
PGF2α - d 7 and 8	33	2.71 ± .15

T-Test μ day 7 = μ day 7 and 8: T = 2.79 df = 34 P<.01

LITERATURE CITED

Abdelgadir, S. E., L. V. Swanson, J. E. Oldfield and F. Stormshak. 1987. Prostaglandin $F2\alpha$ release of oxytocin from bovine corpora lutea in vitro. Biol. Reprod. 37:550.

Abraham, G. E., R. Swerdloff, D. Tulchinsky and W. O. Odell. 1971. Radioimmunoassay of plasma progesterone. J. Clin. Endocrinol. Metab. 32:619.

Adeyemo, O. 1987. Plasma concentration of progesterone during normal estrous cycle and following prostaglandin $F2\alpha$ treatment of Bos indicus and tropic-adapted Bos taurus heifers. Theriogenology 27:759.

Adeyemo, O., U. U. Akpokodje and P. I. Odili. 1979. Control of estrus in Bos indicus and Bos taurus heifers with prostaglandin F2 alpha. Theriogenology 12:255.

Agudo, L. Sp., W. L. Zahler and M. F. Smith. 1984. Effect of prostaglandin $F2\alpha$ on the adenylate cyclase and phosphodiesterase activity of ovine corpora lutea. J. Anim. Sci. 58:955.

Allen, W. R. and L. E. A. Rowson. 1973. Control of the mare's oestrous cycle by prostaglandins. J. Reprod. Fertil. 33:539.

Anderson, L. L., K. P. Bland and R. M. Melampy. 1969. Comparative aspects of uterine-luteal relationships. Rec. Prog. Horm. Res. 25:57.

Anderson, L. L., A. M. Bowerman and R. M. Melampy. 1965. Oxytocin on ovarian function in cycling and hysterectomized heifers. J. Anim. Sci. 24:964.

Anderson, L. L., R. L. Butcher and R. M. Melampy. 1961. Subtotal hysterectomy and ovarian function in gilts. Endocrinology 69:571.

Anderson, L. L., R. L. Butcher and R. L. Melampy. 1963. Uterus and occurrence of oestrus in pigs. Nature 198:311.

Anderson, L. L., F. C. Neal and R. M. Melampy. 1962. Hysterectomy and ovarian function in beef heifers. Amer. J. Vet. Res. 23:794.

Ansotegui, R. P., M. S. Roberson, C. K. Higgins and G. M. Vennes. 1983. Dosage of prostaglandin $F2\alpha$ for estrous synchronization in beef cattle. J. Anim. Sci. 57 (Suppl. 1):383.

Armstrong, D. T. and W. Hansel. 1959. Alteration of the bovine estrous cycle with oxytocin. J. Dairy Sci. 42:533.

Auletta, F. J., D. L. Kamps, S. Pories, J. Bisset and M. Gibson. 1984. An intra-corpus luteum site for the luteolytic action of prostaglandin F2α in the Rhesus monkey. Prostaglandins 27:285.

Babcock, J. C. 1966. Luteotrophic and luteolytic mechanisms in bovine corpora lutea. In: W. Hansel (Ed.) Ovarian Regulatory Mechanisms. p 47. J. Reprod. Fertil. Suppl. 1.

Baird, D. T. and R. B. Land. 1973. Diversion of the uterine vein and the function of the adjacent ovary in the ewe. J. Reprod. Fertil. 33:393.

Baird, D. T. and R. J. Scaramuzzi. 1975. Prostaglandin F2α and luteal regression in the ewe: comparison with 16-aryloxy prostaglandin (ICI 80996). Ann. Biol. Anim. Biochim. Biophys. 15:161.

Barcikowski, B., J. C. Carlson, L. Wilson and J. A. McCracken. 1974. The effect of endogenous and exogenous estradiol-17ß on the release of prostaglandin F2α from the ovine uterus. Endocrinology 95:1340.

Barr, H. L. 1975. Influence of estrus detection on days open in dairy herds. J. Dairy Sci. 58:246.

Barrett, S., M. A. de B. Blockley, J. M. Brown, I. A. Cumming, J. R. Goding, B. J. Mole and J. M. Obst. 1971. Initiation of the oestrous cycle in the ewe by infusions of PGF2α to the auto transplanted ovary. J. Reprod. Fertil. 24:136 (Abstr.).

Bartol, F. F., W. W. Thatcher, G. S. Lewis, E. L. Bliss, M. Drost and F. W. Bazer. 1981. Effect of estradiol-17ß on PGF and total protein concentration in bovine uterine flushings and peripheral plasma concentration of 13,14-dihydro-15-keto-PGF2α. Theriogenology 15:345.

Beavis, E. L. G., J. B. Brown and M. A. Smith. 1969. Ovarian function after hysterectomy with conservation of the ovaries in pre-menopausal women. Brit. Cwlth. J. Obstet. Gynecol. 76:969.

Beavo, J. A., R. S. Hansen, S. A. Harrison, R. L. Hurwitz, T. J. Martins and M. C. Mumby. 1982. Identification and properties of cyclic nucleotide phosphodiesterases. Mol. Cell. Endocrinol. 28:387.

Behrman, H. R., D. L. Grinwich, M. Hichens and G. J. MacDonald. 1978. Effect of hypophysectomy, prolactin, and prostaglandin F2α on gonadotropin binding in vivo and in vitro in the corpus luteum. Endocrinology 103:349.

Behrman, H. R. and M. Hichens. 1976. Rapid block of gonadotropin uptake by corpora lutea in vivo induced by prostaglandin F2α. Prostaglandins 12:83.

Behrman, H. R., J. L. Luborsky-Moore, C. Y. Pang, K. Wright and L. J. Dorflinger. 1979. Mechanism of PGF2α action in functional luteolysis. Adv. Exp. Med. Biol. 112:557.

Behrman, H. R., K. Yoshingaga and R. O. Greep. 1971. Extraluteal effects of prostaglandins. Ann. N. Y. Acad. Sci. 180:426.

Berridge, M. J. 1975. The interaction of cyclic nucleotides and calcium in the control of cellular activity. Adv. Cyclic Nucleotide Res. 14:1.

Black, D. L. and R. T. Duby. 1965. Effect of oxytocin, epinephrine and atropine on the oestrous cycle of the cow. J. Reprod. Fertil. 9:3.

Blatchley, F. R. and B. T. Donovan. 1969. Luteolytic effect of prostaglandin in the guinea pig. Nature 221:1065.

Bolt, D. J. and H. W. Hawk. 1975. Prevention of estrogen-induced regression of corpora lutea in ewes by hysterectomy. J. Anim. Sci. 40:687.

Bosu, W. T. K., P. A. Coig and C. A. V. Barker. 1981. Pregnancy and peripheral plasma progesterone levels in cows inseminated after synchronization of estrus with prostaglandin F2α. Can. Vet. J. 22:59.

Braun, N. S., E. Heath, J. R. Chenault, R. D. Shanks and J. E. Hixon. 1988. Effects of prostaglandin F2α on degranulation of bovine luteal cells on days 4 and 12 of the estrous cycle. Amer. J. Vet. Res. 49:516.

Britt, J. H., H. D. Hafs and J. S. Stevenson. 1978. Estrus in relation to time of administration of prostaglandin F2α to heifers. J. Dairy Sci. 61:513.

Brockett, J. 1977. The American Brahman - Its origination. The Brahman J. 7:6.

Brunner, M. H., L. E. Donaldson and W. Hansel. 1969. Exogenous hormone and luteal function in hysterectomized and intact heifers. J. Dairy Sci. 52:1849.

Buhr, M. M., J. C. Carlson and J. E. Thompson. 1979. A new perspective on the mechanism of corpus luteum regression. Endocrinology 105:1330.

Burford, T. H. and A. W. Diddle. 1956. Effect of total hysterectomy upon the ovary of the macacus rhesus. Surg. Gynecol. Obstet. 62:701.

Burfening, P. J., D. C. Anderson, R. A. Kinkie, J. Williams and R. L. Friedrich. 1978. Synchronization of estrus with PGF2α in beef cattle. J. Anim. Sci. 47:999.

Burns, W. C., M. Koger, A. C. Warnick and C. M. Kincaid. 1959. Beef cattle production data from the West Central Florida Exp. Sta. 1953-1959. Univ. of Fla., Anim. Husb. Nutr. Mimeo. Series 60-5.

Caffrey, J. L., P. W. Fletcher, M. A. Diekman, P. L. O'Callaghan and G. D. Niswender. 1979. The activity of ovine luteal cholesterol esterase during several experimental conditions. Biol. Reprod. 21:601.

Caldwell, B. V. and R. M. Moor. 1971. Further studies on the role of the uterus in the regulation of corpus luteum function in sheep. J. Reprod. Fertil. 26:133.

Caldwell, B. V., S. A. Tillson, W. A. Brock and L. Speroff. 1972. The effects of exogenous progesterone and estradiol on prostaglandin F levels in ovariectomized ewes. Prostaglandins 1:217.

Carlson, J. C., M. M. Buhr and J. C. M. Riley. 1984. Alterations in the cellular membranes of regressing rat corpora lutea. Endocrinology 114:521.

Carlson, J. C., M. M. Buhr, R. Wentworth and W. Hansel. 1982. Evidence of membrane change during regression in the bovine corpus luteum. Endocrinology 110:1472.

Castracane, V. D., G. T. Moore and A. A. Shaikh. 1979. Ovarian function in hysterectomized macaca fascicularis. Biol. Reprod. 20:462.

Channing, C. P. 1972. Stimulatory effects of prostaglandins upon luteinization of Rhesus monkey granulosa cell cultures. Prostaglandins 2:331.

Chegini, N. and C. V. Rao. 1987. Dynamics of nuclear associated granules in bovine luteal cells after treatment in vitro with prostaglandin $F2\alpha$. Endocrinology 121:1870.

Chenault, J. R., W. W. Thatcher, P. S. Kalra, R. M. Abrams and C. J. Wilcox. 1976. Plasma progestins, estradiol, and luteinizing hormone following prostaglandin $F2\alpha$ injection. J. Dairy Sci. 59:1342.

Cheung, W. Y. 1981. Calmodulin and adenylate cyclase-phosphodiesterase system. Cell Calcium 2:263.

Cook, B., F. J. Karsch, D. L. Foster and A. V. Nalbandov. 1974. Estrogen-induced luteolysis in the ewe: possible sites of action. Endocrinology 94:1197.

Cooke, R. G. and A. M. Homeida. 1982. Plasma concentration of 13,14-dihydro-15-keto prostaglandin $F2\alpha$ and progesterone during oxytocin-induced oestrus in the goat. Theriogenology 18:453.

Cooke, R. G. and A. M. Homeida. 1984. Delayed luteolysis and suppression of the pulsatile release of oxytocin after indomethacin treatment in the goat. Res. Vet. Sci. 36:48.

Cooke, R. G. and A. Knifton. 1981. Oxytocin-induced oestrus in the goat. Theriogenology 16:95.

Cooper, M. J. 1974. Control of oestrous cycles of heifers with a synthetic prostaglandin analogue. Vet. Record 95:200.

Coudert, S. P., G. D. Phillips, C. Faiman, W. Chernecki and M. Palmer. 1974a. A study of the utero-ovarian circulation in sheep with reference to local transfer between venous and arterial blood. J. Reprod. Fertil. 36:319.

Coudert, S. P., G. D. Phillips, C. Faiman, W. Chernecki and M. Palmer. 1974b. Infusion of tritiated prostaglandin $F2\alpha$ into the anterior uterine vein of the ewe: absence of local venous-arterial transfer. J. Reprod. Fertil. 36:333.

Coudert, S. P. and R. V. Short. 1966. Prolongation of the functional life of the corpus luteum in sheep with experimental uterine infections. J. Reprod. Fertil. 12:579.

Crockett, J. R., M. Koger and D. E. Franke. 1978. Rotational crossbreeding of beef cattle: reproduction by generation. J. Anim. Sci. 46:1163.

Day, A. M. 1977. Cloprostenol as an aid in dairy herd management: 1. Mating management. N.Z. Vet. J. 25:300.

Del Campo, C. H. and O. J. Ginther. 1972. Anatomy of utero-ovarian vasculature of mares, ewes and sows. J. Anim. Sci. 35:1119.

Del Campo, C. H. and O. J. Ginther. 1973. Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: horses, sheep, and swine. Amer. J. Vet. Res. 34:305.

Dickens, C. 1980. David Copperfield. Franklin Library, Franklin Center, PA.

Diekman, M. A., P. L. O'Callaghan, T. M. Nett and G. D. Niswender. 1978. Effect of prostaglandin $F2\alpha$ on the number of LH receptors in ovine corpus luteum. Biol. Reprod. 19:1010.

Dobson, H. and P. D. G. Dean. 1974. Radioimmunoassay of oestrone, oestradiol-17α and -17β in bovine plasma during the oestrous cycle and last stages of pregnancy. J. Endocrinol. 61:479.

Dobson, H., M. J. Cooper and B. J. A. Furr. 1975. Synchronization of oestrus with ICI 79,939, an analogue of $PGF2\alpha$ and associated changes in plasma progesterone, oestradiol-17β and LH in heifers. J. Reprod. Fertil. 42:141.

Donaldson, L. E. 1977. Synchronisation of oestrus in beef cattle in artificial breeding programs using prostaglandin $F2\alpha$. Aust. Vet. J. 53:72.

Donaldson, L. E., S. P. Glaphin and G. A. Green. 1982. Comparison of two dose rates and two management systems for synchronization of estrus in cattle. Amer. J. Vet. Res. 43:1873.

Dorflinger, L. 1978. Evidence for the role of Ca^{++} in the acute effect of PGF2α on LH-stimulated adenylate cyclase activity. Biol. Reprod. 18 (Suppl. 1):62.

Dorflinger, L. J., P. J. Albert, A. T. Williams and H. R. Behrman. 1984. Calcium is an inhibitor of luteinizing hormone sensitive adenylate cyclase in the luteal cell. Endocrinology 114:1208.

Douglas, R. H., M. R. Del Campo and O. J. Ginther. 1976. Luteolysis following carotid or ovarian arterial injection of prostaglandin F2α in mares. Biol. Reprod. 14:473.

Douglas, R. H. and O. J. Ginther. 1972. Effect of prostaglandin F2α on length of diestrus in mares. Prostaglandins 2:265.

Douglas, R. H. and O. J. Ginther. 1973. Luteolysis following a single injection of prostaglandin F2α in sheep. J. Anim. Sci. 37:990.

Edqvist, L.-E., I. Settergren and G. Åström. 1975. Peripheral plasma levels of progesterone and fertility after prostaglandin F2α induced oestrus in heifers. Cornell Vet. 65:120.

Einer-Jensen, N. and J. A. McCracken. 1981. The transfer of progesterone in the ovarian vascular pedicle of the sheep. Endocrinology 109:685.

Ellicott, A. R., J. R. Hill, Jr. and C. E. Thompson. 1975. Appointment of the hour of mating in the cow. J. Anim. Sci. 41:351 (Abstr.).

Ensminger, M. E. 1976. Beef Cattle Science (5th Ed.). Interstate, Danville.

Erb, R. E., H. A. Gaverick, R. D. Randel, B. L. Brown and C. J. Callahan. 1976. Profiles of reproductive hormones associated with fertile and nonfertile inseminations of dairy cattle. Theriogenology 5:227.

Fairclough, R. J., L. G. Moore, L. T. McGowan, J. F. Smith and W. B. Watkins. 1983. Effect of exogenous progesterone on plasma concentrations of the oxytocin-associated neurophysin and 13,14-dihydro-15-keto-prostaglandin F in intact and ovariectomized ewes over the time of expected luteal regression. Biol. Reprod. 29:271.

Fairclough, R. J., L. G. Moore, A. Peterson and W. B. Watkins. 1984. Effect of oxytocin on plasma concentrations of 13,14-dihydro-15-keto prostaglandin F and the oxytocin-associated neurophysin during the estrous cycle and early pregnancy in the ewe. Biol. Reprod. 31:36.

Fairclough, R. J., J. F. Smith and L. T. McGowan. 1981. Prolongation of the oestrous cycle in cows and ewes after passive immunization with PGF antibodies. J. Reprod. Fertil. 62:213.

Fehr, S., R. Ivell, R. Koll, D. Schams, M. Fields and D. Richter. 1987. Expression of the oxytocin gene in the large cells of the bovine corpus luteum. FEBS Lett. 210:45.

Fields, M. J. and P. A. Fields. 1986. Luteal neurophysin in the nonpregnant cow or ewe: immunological localization in membrane-bounded secretory granules of the large luteal cell. Endocrinology 118:1723.

Fields, M. J., W. B. Watkins, C. M. Barros, and P. A. Field. 1989. Colocalization of oxytocin and neurophysin in secretory granules of the large luteal cell throughout the estrous cycle of the cow. Biol. Reprod. (Submitted).

Fields, P. A., R. K. Eldridge, A. R. Fuchs, R. F. Roberts, and M. J. Fields. 1983. Human placental and bovine corpora luteal oxytocin. Endocrinology 112:1544.

Fitz, T. A., J. L. Fleeger, M. F. Smith and P. G. Harms. 1980. Human chorionic gonadotropin (hCG) binding and adenylate cyclase (AC) activity in developing and regressing corpora lutea (CL). Biol. Reprod. 22 (Suppl. 1):61A.

Fitz, T. A., M. H. Mayan, H. R. Sawyer and G. D. Niswender. 1982. Characterization of two steroidogenic cell types in the ovine corpus luteum. Biol. Reprod. 27:703.

Flint, A. P. F. and E. L. Sheldrick. 1982. Ovarian secretion of oxytocin is stimulated by prostaglandin. Nature 297:587.

Flint, A. P. F. and E. L. Sheldrick. 1983. Evidence for a systemic role for ovarian oxytocin in luteal regression in sheep. J. Reprod. Fertil. 67:215.

Flint, A. P. F. and E. L. Sheldrick. 1985. Continuous infusion of oxytocin prevents induction of uterine oxytocin receptor and blocks luteal regression in cyclic ewes. J. Reprod. Fertil. 75:623.

Flint, A. P. F., E. L. Sheldrick, D. S. C. Jones and F. J. Auletta. 1989. Adaptations to pregnancy in the interactions between luteal oxytocin and the uterus in ruminants. In: M. Shemesh and B. Weir (Eds.) Maternal Recognition of Pregnancy and Maintenance of the Corpus Luteum. p 196. J. Reprod. Fertil. Suppl. 37.

Fogwell, R. L., J. L. Cowley, J. A. Wortman, N. K. Ames and J. J. Ireland. 1985. Luteal function in cows following destruction of ovarian follicles at midcycle. Theriogenology 23:389.

Folman, Y., M. Rosenberg, Z. Herz and M. Davidson. 1973. The relationship between plasma progesterone concentration and conception in post-partum dairy cows maintained on two levels of nutrition. J. Reprod. Fertil. 34:267.

Fonseca, F. A., J. H. Britt, B. T. McDaniel, J. C. Wilk and A. H. Rakes. 1983. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. J. Dairy Sci. 66:1128.

Foote, R. H. 1986. Artificial insemination. In: S. J. Roberts (Ed.) Veterinary Obstetrics and Genital Diseases (Theriogenology). Foote, Woodstock.

Fowler, S. H. 1969. Beef production in the south. Interstate, Danville.

Frank, M., F. W. Bazer, W. W. Thatcher and C. J. Wilcox. 1978. A study of prostaglandin $F2_\alpha$ as the luteolysin in swine: IV. An explanation for the luteotrophic effect of estradiol. Prostaglandins 15:151.

Galina, C. S., A. Calderón and M. McCloskey. 1982. Detection of signs of estrus in the Charolais cow and its Brahman cross under continuous observation. Theriogenology 17:485.

Garverick, H. A., M. F. Smith, R. G. Elmore, G. L. Morehouse, L. S. Agudo and W. L. Zahler. 1985. Changes and interrelationships among luteal LH receptors, adenylate cyclase activity and phosphodiesterase activity during the bovine estrous cycle. J. Anim. Sci. 61:216.

Gemmel, R. T. and B. D. Stacy. 1979. Ultrastructural study of granules in the corpora lutea of several mammalian species. Amer. J. Anat. 155:1.

Gemmel, R. T., B. D. Stacy and G. D. Thorburn. 1974. Ultrastructural study of the secretory granules in the corpus luteum of the sheep during the estrous cycle. Biol. Reprod. 11:447.

Gilson, W. D., G. M. Hill, C. R. Looney and R. A. Godke. 1981. Synchronizing estrus in Brahman based cattle using Lutalyse and inseminating at 12 hours post-estrus or 80 hours post-injection. J. Anim. Sci. 53 (Suppl. 1):58.

Ginther, O. J. 1968. Utero-ovarian relationships in cattle: applied veterinary aspects. J. Amer. Vet. Med. Assoc. 153:1665.

Ginther, O. J. 1971. Response of corpora lutea to cauterization of follicles in sheep. Amer. J. Vet. Res. 32:59.

Ginther, O. J. 1974. Internal regulation of physiological processes through local venoarterial pathways: A review. J. Anim. Sci. 39:550.

Ginther, O. J. and G. E. Bisgard. 1972. Role of the main uterine vein in local action of an intrauterine device on the corpus luteum in sheep. Amer. J. Vet. Res. 33:1583.

Ginther, O. J. and C. H. Del Campo. 1974. Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: Cattle. Am. J. Vet. Res. 35:193.

Ginther, O. J., C. H. Del Campo and C. A. Rawlings. 1973. Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: A local venoarterial pathway between uterus and ovaries in sheep. Am. J. Vet. Res. 34:723.

Ginther, O. J. and N. L. First. 1971. Maintenance of the corpus luteum in hysterectomized mares. Amer. J. Vet. Res. 32:1687.

Ginther, O. J., A. L. Pope and L. E. Casida. 1966a. Local effect of an intrauterine plastic coil on the corpus luteum of the ewe. J. Anim. Sci. 25:472.

Ginther, O. J., C. O. Woody, K. Janakiraman and L. E. Casida. 1966b. Effect of an intra-uterine plastic coil on the oestrous cycle of the heifer. J. Reprod. Fertil. 12:193.

Ginther, O. J., C. O. Woody, S. Mahajan, K. Janakiraman and L. E. Casida. 1967. Effect of oxytocin administration on the oestrous cycle of unilaterally hysterectomized heifers. J. Reprod. Fertil. 14:225.

Glencross, R. G. and G. S. Pope. 1981. Concentrations of oestradiol-17β and progesterone in the plasma of dairy heifers before and after cloprostenol-induced and natural luteolysis and during early pregnancy. Anim. Reprod. Sci. 4:93.

Goding, J. R., F. A. Harrison, R. B. Heap and J. L. Linzell. 1967. Ovarian activity in the ewe after auto transplantation of the ovary or uterus to the neck. J. Physiol. 191:129P (Abstr.).

Gonzalez, L. V., J. W. Fuquay and H. J. Bearden. 1985. Insemination management for a one-injection prostaglandin $F2_{\alpha}$ synchronization regimen. I. One daily insemination period versus use of the A.M./P.M. rule. Theriogenology 24:495.

Goodsaid-Zalduondo, F., D. A. Rintoul, J. C. Carlson and W. Hansel. 1982. Luteolysis-induced changes in phase composition and fluidity of bovine luteal cell membranes. Proc. Natl. Acad. Sci. 79:4332.

Gore, S. D. and H. R. Behrman. 1984. Alteration of transmembrane sodium and potassium gradients inhibits the action of luteinizing hormone in the luteal cell. Endocrinology 114:2020.

Graves, R. L., R. G. Lutz, J. W. Riesen, T. A. Hoagland and C. O. Woody. 1985. Factors influencing estrus and conception in dairy heifers after prostaglandin F2-alpha. Theriogenology 23:733.

Greenstein, J. S., R. W. Murray and R. C. Foley. 1958. Effect of exogenous hormones on the reproductive processes of the cycling dairy heifer. J. Dairy Sci. 41:1834 (Abstr.).

Grinwich, D. L., M. Hichens and H. R. Behrman. 1976. Control of the LH receptor by prolactin and prostaglandin F2α in rat corpora lutea. Biol. Reprod. 14:212.

Guldenaar, S. E. F., D. C. Wathes and B. T. Pickering. 1984. Immunocytochemical evidence for the presence of oxytocin and neurophysin in the large cells of the bovine corpus luteum. Cell Tissue Res. 237:349.

Gutknecht, G. D., J. C. Cornette and B. B. Pharriss. 1969. Antifertility properties of prostaglandin F2α. Biol. Reprod. 1:367.

Hafez, E. S. E. 1987. Folliculogenesis, egg maturation and ovulation. In: Reproduction in Farm Animals. p 156. Lea and Febinger, Philadelphia.

Hafs, H. D. and J. G. Manns. 1975. Onset of oestrus after prostaglandin F2α in cattle. Vet. Record 96:134.

Hafs, H. D., J. G. Manns and G. E. Lamming. 1975. Fertility of cattle from AI after PGF2α. J. Anim. Sci. 41:355 (Abstr.).

Hagen, D. R., P. A. Martin and P. J. Dziuk. 1981. Effect of ovarian autotransplantation to various locations on estrual cyclicity in gilts. Biol. Reprod. 25:359.

Hamberger, L., L. Nilsson, B. Dennefors, I. Khan and A. Sjoegren. 1979. Cyclic AMP formation of isolated human corpora lutea in response to hCG-interference by PGF2α. Prostaglandins 17:615.

Hansel, W. and E. M. Convey. 1983. Physiology of the estrous cycle. J. Anim. Sci. 57 (Suppl. 2):404.

Hansel, W. and S. E. Echternkamp. 1972. Control of ovarian function in domestic animals. Am. Zoologist 12:225.

Hansel, W. and R. B. Snook. 1970. Pituitary ovarian relationships in the cow. J. Dairy Sci. 53:945.

Hansel, W. and W. C. Wagner. 1960. Luteal inhibition in the bovine as a result of oxytocin injections, uterine dilation, and intrauterine infusions of seminal and preputial fluids. J. Dairy Sci. 43:796.

Hansen, T. R., R. D. Randel and L. A. Peterson. 1987a. Bovine corpus luteum regression and estrous response following treatment with alfaprostol. J. Anim. Sci. 64:1280.

Hansen, T. T., R. D. Randel, E. C. Segerson, Jr., L. M. Rutter and P. G. Harms. 1987b. Corpus luteum function following spontaneous or prostaglandin-induced estrus in Brahman cows and heifers. J. Anim. Sci. 65:524.

Haour, F. and B. B. Saxena. 1974. Characterization and solubilization of gonadotropin receptor of bovine corpus luteum. J. Biol. Chem. 249:2195.

Hardin, D. R. and R. D. Randel. 1982. The effect of cloprostenol and cloprostenol plus hCG on corpora lutea and serum progesterone in Brahman cows. Theriogenology 17:669.

Hardin, D. R., A. C. Warnick, T. H. Wise, R. H. Schultz and M. J. Fields. 1980a. Artificial insemination of subtropical commercial beef cattle following synchronization with cloprostenol (ICI 80996): I. Fertility. Theriogenology 14:249.

Hardin, D. R., A. C. Warnick and M. J. Fields. 1980b. Artificial insemination of subtropical commercial beef cattle following synchronization with cloprostenol (ICI 80996): II. Estrous response. Theriogenology 14:259.

Harrison, L. M., N. Kenny and G. D. Niswender. 1987. Progesterone production, LH receptors, and oxytocin secretion by ovine luteal cell types on days 6, 10 and 15 of the estrous cycle and day 25 of pregnancy. J. Reprod. Fertil. 79:539.

Harrison, L. M., R. D. Randel, D. W. Forest, J. G. Betts, W. D. Humphrey and D. R. Hardin. 1985. Cloprostenol induced luteal regression in the beef cow. I. Ovarian response and endocrine changes. Theriogenology 23:511.

Hawk, H. W. 1970. Physiologic responses to conception control methods in domestic animals. J. Am. Vet. Med. Assoc. 157:1795.

Hawk, H. W. and D. J. Bolt. 1970. Luteolytic effect of estradiol-17ß when administered after midcycle in the ewe. Biol. Reprod. 2:275.

Heath, E., P. Weinstein, B. Merritt, R. Shanks and J. Hixon. 1983. Effects of prostaglandins on the bovine corpus luteum: granules, lipid inclusions and progesterone secretion. Biol. Reprod. 29:977.

Henderson, K. M. and K. P. McNatty. 1975. A biochemical hypothesis to explain the mechanism of luteal regression. Prostaglandins 9:779.

Henricks, D. M., J. F. Dickey and G. D. Niswender. 1970. Serum luteinizing hormone and plasma progesterone levels during the estrous cycle and early pregnancy in cows. Biol. Reprod. 2:346.

Henricks, D. M., D. R. Lamond, J. R. Hill and J. F. Dickey. 1971. Plasma progesterone concentrations before mating and in early pregnancy in the beef heifer. J. Anim. Sci. 33:450.

Henricks, D. M., J. T. Long, J. R. Hill and J. F. Dickey. 1974. The effect of prostaglandin F2α during various stages of the oestrous cycle of beef heifers. J. Reprod. Fertil. 41:113.

Hichens, M., D. L. Grinwich and H. R. Behrman. 1974. PGF2α-induced loss of corpus luteum gonadotrophin receptors. Prostaglandins 7:449.

Higgins, C. K., E. L. Moody, D. K. Han, R. P. Ansotegui and P. J. Burfening. 1981. Reproductive and productive performance of PGF2α-synchronized and non-synchronized beef cattle. J. Anim. Sci. 53 (Suppl. 1):501.

Hirata, F. and J. Axelrod. 1980. Phospholipid methylation and biological signal transmission. Science 209:1082.

Hirst, J. J., G. E. Rice, G. Jenkin and G. D. Thorburn. 1986. Secretion of oxytocin and progesterone by ovine corpora lutea in vitro. Biol. Reprod. 35:1106.

Hirst, J. J., G. E. Rice, G. Jenkin and G. D. Thorburn. 1988. Control of oxytocin secretion by ovine corpora lutea: Effects of arachidonic acid, phospholipases, and prostaglandins. Endocrinology 122:774.

Hixon, J. E. and A. P. F. Flint. 1987. Effects of a luteolytic dose of oestradiol benzoate on uterine oxytocin receptor concentrations, phosphoinostide turnover, and prostaglandin F2α secretion in sheep. J. Reprod. Fertil. 79:457.

Hixon, J. E., D. R. Gengenbach and W. Hansel. 1975. Failure of prostaglandin F2α to cause luteal regression in ewes after destruction of ovarian follicles by x-irradiation. Biol. Reprod. 13:126.

Hixon, J. E. and W. Hansel. 1974. Evidence for preferential transfer of prostaglandin F2α to the ovarian artery following intrauterine administration in cattle. Biol. Reprod. 11:543.

Hixon, J. E. and W. Hansel. 1979. Effects of prostaglandin F2α, estradiol, and luteinizing hormone in dispersed cell preparations of bovine corpora lutea. Adv. Exp. Med. Biol. 112:613.

Hixon, J. E., C. A. Pimentel, P. G. Weston, E. P. Chafetz, R. D. Shanks and W. Hansel. 1983. A luteolytic interaction between estradiol benzoate and prostaglandin F2α in cattle. J. Anim. Sci. 56:1190.

Hooper, S. B., W. B. Watkins, and G. D. Thorburn. 1986. Oxytocin, oxytocin-associated neurophysin, and prostaglandin F2α concentrations in the utero-ovarian vein of pregnant and nonpregnant sheep. Endocrinology 119:2590.

Inskeep, E. K. 1973. Potential uses of prostaglandins in control of reproductive cycles of domestic animals. J. Anim. Sci. 36:1149.

Inskeep, E. K. and R. L. Butcher. 1966. Local component of utero-ovarian relationships in the ewe. J. Anim. Sci. 25:1164.

Irvin, H. J., R. D. Randel, W. E. Haensly and A. M. Sorensen, Jr. 1978. Reproductive studies of Brahman cattle III. Comparison of weight, progesterone content, histological characteristics, and 3β-hydroxy-steroid dehydrogenase activity in corpora lutea of Brahman, Hereford, and Brahman X Hereford heifers. Theriogenology 10:417.

Ivell, R., K. H. Brackett, M. J. Fields and D. Richter. 1985. Ovulation triggers oxytocin gene expression in the bovine ovary. FEBS Letters 190:263.

Ivell, R. and D. Richter. 1984. The gene for the hypothalamic peptide hormone oxytocin is highly expressed in the bovine corpus luteum: Biosynthesis, structure and sequence analysis. EMBO J. 3:2351.

Jackson, P. S., C. T. Johnson, B. J. Furr and J. F. Beattie. 1979. Influence of stage of oestrous cycle on time of oestrus following cloprostenol treatment in the bovine. Theriogenology 12:153.

Jiménez, F., C. S. Galina, B. Ramirez and R. Navarro-Fierro. 1985. Comparative study of the concentrations of peripheral progesterone before and after PGF2 alpha injection between Bos taurus (Brown Swiss) and Bos indicus (Indubrazil) in the tropics. Anim. Rep. Sci. 9:333.

Jöchle, W., D. Kuzmanov and J. Vujosevic. 1982. Estrous cycle synchronization in dairy heifers with the prostaglandin analog alphaprostol (I). Theriogenology 18:215.

Johnson, C. T. 1978. Time of onset of oestrus after the injection of heifers with cloprostenol. Vet. Record 103:204.

Kaltenbach, C. C., J. W. Graber, G. D. Niswender and A. V. Nalbandov. 1968. Luteotrophic properties of some pituitary hormones in nonpregnant and pregnant hypophysectomized ewes. Endocrinology 82:818.

Kaltenbach, C. C., G. D. Niswender, D. R. Zimmerman and J. N. Wiltbank. 1964. Alteration of ovarian activity in cycling, pregnant and hysterectomized heifers with exogenous estrogens. J. Anim. Sci. 23:995.

Kamonpatana, M., A. Kunawongkrit, P. Bodhipaksha and Y. Luvira. 1979. Effect of PGF-2α on serum progesterone levels in the swamp buffalo (Bubalus bubalis). J. Reprod. Fertil. 56:445.

Katz, R. L. and G. J. Katz. 1974. Prostaglandins: basic and clinical considerations. Anesthesiology 40:471.

Kazmer, G. W., M. A. Barnes, R. D. Halman and J. F. Dickey. 1981. Endogenous hormone response and fertility in dairy heifers after treatment with Lutalyse and GnRH. Theriogenology 16:575.

Kincaid, C. M. 1962. Breed crosses with beef cattle in the South. Southern Cooperative Series Bull. 81, Fla. Agr. Exp. Sta.

Kindahl, H., L.-E. Edquist, A. Bane and E. Granström. 1976a. Blood levels of progesterone and 15-keto-13,14-dehydro-prostaglandin $F2\alpha$ during the normal oestrous cycle and early pregnancy in heifers. Acta Endocrinol. 82:134.

Kindahl, H., L.-E. Edquist, E. Granström, and A. Bane. 1976b. The release of prostaglandin $F2\alpha$ as reflected by 15-keto-13,14-dihydroprostaglandin $F2\alpha$ in the peripheral circulation during normal luteolysis in heifers. Prostaglandins 11:871.

King, G. J. and H. A. Robertson. 1974. A two injection schedule with prostaglandin $F2\alpha$ for the regulation of the ovulatory cycle in cattle. Theriogenology 1:123.

King, M. E., G. H. Kiracofe, J. S. Stevenson and R. R. Schalles. 1982. Effect of stage of the estrous cycle on interval to estrus $PGF2\alpha$ in beef cattle. Theriogenology 18:191.

Kiracofe, G. H., C. S. Menzies, H. T. Gier and H. G. Spies. 1966. Effect of uterine extracts and uterine or ovarian blood vessel ligations on ovarian function of ewes. J. Anim. Sci. 25:1159.

Kiracofe, G. H., L. E. Keay and K. G. Odde. 1985. Synchronization of estrus in cyclic beef heifers with the prostaglandin analog alphaprostol. Theriogenology 24:737.

Kirton, K. T., B. B. Pharriss and A. D. Forbes. 1970. Luteolytic effects of prostaglandin $F2\alpha$ in primates. Proc. Soc. Exp. Biol. Med. 133:314.

Knickerbocker, J. J., W. W. Thatcher, D. B. Foster, D. Wolfenson, F. F. Bartol and D. Caton. 1986. Uterine prostaglandin and blood flow responses to estradiol-17ß in cyclic cattle. Prostaglandins 31:757.

Koch, Y., J. Zor, P. Chobsieng, S. A. Lamprecht, S. Pomerantz and H. R. Lindler. 1974. Binding of luteinizing hormone and human chorionic gonadotrophin to ovarian cells and activation of adenylate cyclase. J. Endocrinol. 61:179.

Koering, M. J. and K. T. Kirton. 1973. The effects of prostaglandin $F2\alpha$ on the structure and function of the rabbit ovary. Biol. Reprod. 9:226.

Koger, M., T. J. Cunha and A. C. Warnick (Ed.). 1973. Crossbreeding Beef Cattle, Series 2. Univ. of Fla. Press, Gainesville.

Koos, R. D. and W. Hansel. 1981. The large and small cells of the bovine corpus luteum: Ultrastructural and functional differences. In: N. B. Swartz and M. Hunzicker-Dunn (Eds.) Dynamics of Ovarian Function. pp 197-203. Raven Press, New York.

Kotwica, J. D., D. Schams, H. H. D. Meyer and Th. Mittermeier. 1988. Effect of continuous infusion of oxytocin on length of the oestrous cycle and luteolysis in cattle. J. Reprod. Fertil. 83:287.

Kruip, Th. A. M., H. G. B. Vullings, D. Schams, J. Jonis and A. Klarenbeek. 1985. Immunocytochemical demonstration of oxytocin in bovine ovarian tissues. Acta Endocrinol. 109:537.

Kurzrok, R. and C. C. Lieb. 1930. Biochemical studies of human semen. II. The action of semen on the human uterus. Proc. Soc. Exp. Biol. Med. 28:268.

Lafrance, M. and A. K. Goff. 1985. Effect of pregnancy on oxytocin-induced release of prostaglandin $F2\alpha$ in heifers. Biol. Reprod. 33:1113.

Lafrance, M. and A. K. Goff. 1988. Effects of progesterone and oestradiol-17β on oxytocin-induced release of prostaglandin $F2\alpha$ in heifers. J. Reprod. Fertil. 82:429.

Lahav, M., A. Freud and H. R. Lindner. 1976. Abrogation by prostaglandin $F2\alpha$ of LH-stimulated cyclic AMP accumulation in isolated rat corpora lutea of pregnancy. Biochem. Biophys. Res. Comm. 68:1294.

Lambert, P. W., W. M. Greene, J. D. Strickland, D. K. Han and E. L. Moody. 1976. $PGF2\alpha$ controlled estrus in beef cattle. J. Anim. Sci. 42:1565 (Abstr.).

Lamond, D. R. and M. Drost. 1973. The counter-current transfer of prostaglandin in the ewe. Prostaglandins 3:691.

Lamond, D. R., R. V. Tomlinson, M. Drost, D. M. Henricks and W. Jöchle. 1973. Studies of prostaglandin $F2\alpha$ in the cow. Prostaglandins 4:269.

Landivar, C., C. S. Galina, A. Duchateau and R. Navarro-Fierro. 1985. Fertility trial in Zebu cattle after a natural or controlled estrus with prostaglandin F2 alpha, comparing natural mating with artificial insemination. Theriogenology 23:421.

Lauderdale, J. W., J. F. McAllister, D. D. Kratzer and E. L. Moody. 1981. Use of prostaglandin $F2\alpha$ ($PGF2\alpha$) in cattle breeding. ACTA Vet. Scand. Suppl. 77:181.

Lauderdale, J. W., J. F. McAllister, E. L. Moody and D. D. Kratzer. 1980. Pregnancy rate in beef cattle injected once with $PGF2\alpha$. J. Anim. Sci. 51 (Suppl. 1):296.

Lauderdale, J. W., B. E. Seguin, J. N. Stellflug, J. R. Chenault, W. W. Thatcher, C. K. Vincent and A. F. Loyancano. 1974. Fertility of cattle following PGF2α injection. J. Anim. Sci. 38:964.

LaVoie, V. A., G. R. Poncelet, D. K. Han, C. L. Soliday, P. W. Lambert and E. L. Moody. 1975. Effect of prostaglandin F2α on the estrous cycle, corpora lutea and progesterone levels of hysterectomized cows. J. Anim. Sci. 41:166.

Leaver, J. D., R. G. Glencross and G. S. Pope. 1975. Fertility of Friesian heifers after luteolysis with prostaglandin analogue ICI 80996. Vet. Record 96:383.

Leavitt, W. W., W. C. Okulicz, J. A. McCracken, W. Schramm and W. F. Robidoux, Jr. 1985. Rapid recovery of nuclear estrogen receptor and oxytocin receptor in the ovine uterus following progesterone withdrawal. J. Steroid Biochem. 22:687.

Lehmann, F., F. Peters, M. Breckwoldt and G. Bettendorf. 1972. Plasma progesterone levels during infusion of prostaglandin F2α. Prostaglandins 1:269.

Lewis, P. E. and J. E. Warren, Jr. 1977. Effect of indomethacin on luteal function in ewes and heifers. J. Anim. Sci. 46:763.

Loeb, L. 1923. The effect of extirpation of the uterus on the life and function of the corpus luteum in the guinea pig. Proc. Soc. Exp. Biol. Med. 20:441.

Loeb, L. 1927. The effects of hysterectomy on the system of sex organs and on the periodicity of the sexual cycle in the guinea pig. Amer. J. Physiol. 83:202.

Lopez-Barbella, S. R., A. C. Warnick, T. H. Wise and M. J. Fields. 1979. Endocrine response of the cow to PMSG and subsequent multiple corpora lutea regression by prostaglandin F2α. J. Anim. Sci. 48:1135.

Louis, T. M., H. D. Hafs and D. A. Morrow. 1974. Intrauterine administration of prostaglandin F2α in cows: Progesterone, estrogen, LH, estrus and ovulation. J. Anim. Sci. 38:347.

Louis, T. M., H. D. Hafs and B. E. Seguin. 1973. Progesterone, LH, estrous and ovulation after prostaglandin F2α in heifers. Proc. Soc. Exp. Biol. Med. 143:152.

Lucy, M. C., J. S. Stevenson and E. P. Call. 1986. Controlling first service and calving interval by prostaglandin F2α, gonadotrophin-releasing hormone, and timed insemination. J. Dairy Sci. 69:2186.

Lukaszewska, J., L. Wilson, Jr. and W. Hansel. 1972. Luteotropic and luteolytic effects of prostaglandins in the hamster. Proc. Soc. Exp. Biol. Med. 140:1302.

Macmillan, K. L. 1978. Oestrus synchronization with a prostaglandin analogue. III. Special aspects of synchronization. N.Z. Vet. J. 26:104.

Macmillan, K. L. 1983. Prostaglandin responses in dairy herd breeding programmes. N.Z. Vet. J. 31:110.

Macmillan, K. L. and A. M. Day. 1982. Prostaglandin F2α - A fertility drug in dairy cattle. Theriogenology 18:245.

Madan, M. L. and H. D. Johnson. 1973. Environmental heat effects on bovine luteinizing hormone. J. Dairy Sci. 56:1420.

Maffeo, G., R. Ballabio, V. Olgiati and F. Guidobono. 1983. Induction of estrus in cows by a new analogue of PGF2α (alfaprostol). Prostaglandins 25:541.

Manns, J. G., H. D. Hafs and G. E. Lamming. 1975. Influence of thyrotropin releasing hormone (TRH) on plasma progesterone and pituitary hormone concentrations in cattle. Can. J. Anim. Sci. 55:633.

Manns, J. G., M. S. Wenkoff and W. M. Adams. 1976. Time of AI on fertility of heifers treated with PGF2α. J. Anim. Sci. 43:295 (Abstr.).

Mapletoft, R. J., M. R. Del Campo and O. J. Ginther. 1975. Unilateral luteotropic effect of uterine venous effluent of a gravid uterine horn in sheep. Proc. Soc. Exp. Biol. Med. 150:129.

Mapletoft, R. J., M. R. Del Campo and O. J. Ginther. 1976. Local venoarterial pathway for uterine-induced luteolysis in cows. Proc. Soc. Exp. Biol. Med. 153:289.

Mapletoft, R. J. and O. J. Ginther. 1975. Adequacy of main uterine vein and the ovarian artery in the local venoarterial pathway for uterine-induced luteolysis in ewes. Am. J. Vet. Res. 36:957.

Marsh, J. M. 1970. The stimulatory effect of luteinizing hormone on adenyl cyclase in the bovine corpus luteum. J. Biol. Chem. 245:1596.

Marsh, J. M. 1971. The effect of prostaglandins on the adenyl cyclase of the bovine corpus luteum. Ann. N. Y. Acad. Sci. 180:416.

Maurer, R. R. and S. E. Echternkamp. 1982. Hormonal asynchrony and embryonic development. Theriogenology 17:11.

McCracken, J. A., D. T. Baird and J. R. Goding. 1971. Factors affecting the secretion of steroids from the transplanted ovary in the sheep. Rec. Prog. Horm. Res. 27:537.

McCracken, J. A., J. C. Carlson, M. E. Glew, J. R. Goding, D. T. Baird, K. Green and B. Samuelsson. 1972. Prostaglandin F2α identified as a luteolytic hormone in sheep. Nature 238:129.

McCracken, J. A. and N. Einer-Jensen. 1979. Prostaglandin F2α and its 13-dehydroanalogs: comparative luteolytic effects in vivo. Adv. Exp. Med. Biol. 112:577.

McCracken, J. A., M. E. Glew and R. J. Scaramuzzi. 1970. Corpus luteum regression induced by prostaglandin F2α. J. Clin. Endocrinol. Metab. 30:544.

McCracken, J. A., W. Schramm and W. C. Okulicz. 1984. Hormone receptor control of pulsatile secretion of PGF2α from the ovine uterus during luteolysis and its abrogation in early pregnancy. Anim. Reprod. Sci. 7:31.

Meyer, H. H. D., Th. Mittermeier and D. Schams. 1988. Dynamics of oxytocin, estrogen and progestogen receptors in the bovine endometrium during the estrous cycle. Acta Endocrinol. 118:96.

Milvae, R. A., H. W. Alila and W. Hansel. 1983. Methylation in bovine luteal cells as a regulator of luteinizing hormone action. Biol. Reprod. 29:849.

Milvae, R. A. and W. Hansel. 1980. Concurrent uterine venous and ovarian arterial prostaglandin F concentrations in heifers treated with oxytocin. J. Reprod. Fertil. 60:7.

Moeljono, M. P., F. W. Bazer and W. W. Thatcher. 1976. A study of prostaglandin F2α as the luteolysin in swine: I. Effect of prostaglandin F2α in hysterectomized gilts. Prostaglandins 11:737.

Moody, E. L. and J. W. Lauderdale. 1977. Fertility of cattle following PGF2α controlled ovulation. J. Anim. Sci. 46 (Suppl. 1): 415.

Moor, R. M. and L. A. Rowson. 1964. Influence of the embryo and uterus on luteal function in the sheep. Nature 201:522.

Moor, R. M. and L. E. A. Rowson. 1966. Local uterine mechanisms affecting luteal function in the sheep. J. Reprod. Fertil. 11:307.

Moore, L. G., V. J. Choy, R. L. Elliott and W. B. Watkins. 1986. Evidence for the pulsatile release of PGF2α inducing the release of ovarian oxytocin during luteolysis in the ewe. J. Reprod. Fertil. 76:159.

Moore, W. W. and A. V. Nalbandov. 1953. Neurogenic effects of uterine distension on the estrous cycle of the ewe. Endocrinology 53:1.

Moreno, I. Y. D., C. S. Galina, F. J. Escobar, B. Ramirez and R. Navarro-Fierro. 1986. Evaluation of the lytic response of prostaglandin F2 alpha in zebu cattle based on serum progesterone. Theriogenology 25:413.

Moskowitz, N., L. Shapiro, W. Schook and S. Puszkin. 1983. Phospholipase A2 modulation by calmodulin, prostaglandins, and cyclic nucleotides. Biochem. Biophys. Res. Comm. 115:94.

Nagaratnam, V., T. Sooriyamoorthy, E. O. Oyedipe and A. Y Zakari. 1983. Synchronization of oestrus with cloprostenol and subsequent calving rates in artificially inseminated zebu heifers. Br. Vet. J. 139:440.

Nett, T. M., M. C. McClellan and G. D. Niswender. 1976. Effects of prostaglandins on the ovine corpus luteum: Blood flow, secretion of progesterone and morphology. Biol. Reprod. 15:66.

Neuendorff, D. A., R. D. Randel and J. W. Lauderdale. 1984. Efficacy of Lutalyse for estrous synchronization in Brahman cattle. J. Anim. Sci. 59 (Suppl. 1):14.

Newcomb, R., W. D. Booth and L. E. A. Rowson. 1977. The effect of oxytocin treatment on the levels of prostaglandin F in the blood of heifers. J. Reprod. Fertil. 49:17.

Niswender, G. D., P. J. Dziuk, J. Graber and C. C. Kaltenbach. 1970. Function of the corpus luteum in the ewe following relocation of the uterus or embryo. J. Anim. Sci. 30:935.

Niswender, G. D., T. J. Reimers, M. A. Diekman and T. M. Nett. 1976. Blood flow: A mediator of ovarian function. Biol. Reprod. 14:64.

O'Grady, J. P., E. I. Kohorn, R. H. Glass, B. V. Caldwell, W. A. Brock and L. Speroff. 1972. Inhibition of progesterone synthesis in vitro by prostaglandin $F2\alpha$. J. Reprod. Fertil. 30:153.

Olds, D., T. Cooper and F. A. Thrift. 1979. Effects of days open on economic aspects of current lactation. J. Dairy Sci. 62:1167.

Orihuela, A., C. Galina, J. Escobar and E. Riquelme. 1983. Estrous behavior following prostaglandin $F2\alpha$ injection in zebu cattle under continuous observation. Theriogenology 19:795.

Ott, I. and J. C. Scott. 1910. The galactogogue action of the thymus and corpus luteum. Proc. Soc. Exp. Biol. Med. 8:49.

Oxender, W. D., P. A. Noden, T. M. Louis and H. D. Hafs. 1974. A review of prostaglandin $F2\alpha$ for ovulation control in cows and mares. Am. J. Vet. Res. 35:997.

Oxenreider, S. L., R. C. McClure and B. N. Day. 1965. Arteries and veins of the internal genitalia of female swine. J. Reprod. Fertil. 9:19.

Oyedipe, E. O., B. Gustafassen and H. Kindahl. 1984. Blood levels of progesterone and 15-keto-13,14-dihydro prostaglandin F2α during the estrous cycle of oxytocin treated cows. Theriogenology 22:329.

Pate, J. L. and W. A. Condon. 1984. Effects of prostaglandin F2α on agonist-induced progesterone production in cultured bovine luteal cells. Biol. Reprod. 31:427.

Patek, C. E. and J. Watson. 1976. Prostaglandin production during the oestrous cycle by porcine and ovine corpus luteum tissue. J. Endocrinol. 71:47p.

Peterson, A. J., R. J. Fairclough, E. Payne and J. F. Smith. 1975. Hormonal changes around bovine luteolysis. Prostaglandins 10:675.

Pexton, J. E., S. P. Ford, L. Wilson, Jr., R. L. Butcher and E. K. Inskeep. 1975. Prostaglandins F in uterine tissue and venous plasma of ewes with intrauterine devices. J. Anim. Sci. 41:144.

Pharriss, B. B. and L. J. Wyngarden. 1969. The effect of prostaglandin F2α on the progestogen content of ovaries from pseudopregnant rats. Proc. Soc. Exp. Biol. Med. 130:92.

Pharriss, B. B., L. J. Wyngarden and G. D. Gutknecht. 1968. Biological interactions between prostaglandins and luteotropins in the rat. In: E. Rosemberg (Ed.) Gonadotropins. pp 121-129. Geron, Los Altos.

Pierson, R. A. and O. J. Ginther. 1984. Ultrasonography of the bovine ovary. Theriogenology 21:495.

Piper, P. J., J. R. Vane and J. H. Wyllie. 1970. Inactivation of prostaglandins by the lungs. Nature 225:600.

Plunkett, S. S., J. S. Stevenson and E. P. Call. 1984. Prostaglandin F2α for lactating dairy cows with a palpable corpus luteum but unobserved estrus. J. Dairy Sci. 67:380.

Powell, W. S., S. Hammarstroem and B. Samuelsson. 1974. Prostaglandin F2α receptor in ovine corpora lutea. Eur. J. Biochem. 41:103.

Poyser, N. L., E. W. Horton, C. J. Thompson and M. Los. 1971. Identification of prostaglandin F2α release by distension of guinea pig uterus in vitro. Nature 230:526.

Priedkalns, J. and A. F. Weber. 1968. Ultrastructural studies of the bovine Graafian follicle and corpus luteum. Z. Zellforsch Mikrosk Anat. 91:554.

Quirk, S. J., D. L. Willcox, D. M. Parry and G. D. Thorburn. 1979. Subcellular location of progesterone in the bovine corpus luteum: A biochemical, morphological and cytochemical investigation. Biol. Reprod. 20:1133.

Randel, R. D. 1984. Seasonal effects on female reproductive functions in the bovine (Indian breeds). Theriogenology 21:170.

Randel, R. D. R. W. Godfrey, L. A. Peterson and J. S. Bluntzer. 1984. Comparison of Alfaprostol and Lutalyse for estrus synchronization of Brahman and Brangus cattle. J. Anim. Sci. 59 (Suppl. 1):12.

Rao, C. V. 1975. The presence of discrete receptors for prostaglandin F2α in the cell membranes of bovine corpora lutea. Biochem. Biophys. Res. Comm. 64:416.

Rao, C. V. 1976. Inhibition of [^{3}H] prostaglandin F2α binding to its receptors by progesterone. Steroids 27:831.

Rao, C. V., V. L. Estergreen, F. R. Carman, Jr. and G. E. Moss. 1979. Receptors for gonadotrophin and prostaglandin F2α in bovine corpora lutea of early, mid, and late luteal phase. Acta Endocrinol. 91:529.

Rao, C. V., J. J. Ireland and J. F. Roche. 1984. Decrease of various luteal enzyme activities during prostaglandin F2α-induced luteal regression in the bovine. Mol. Cell. Endocrinol. 34:99.

Refsal, K. R. and B. E. Seguin. 1980. Effect of stage of diestrus and number of cloprostenol (ICI 80,996) injections on intervals to estrus, LH peak and ovulation in heifers. Theriogenology 14:37.

Remsen, L. G. and J. D. Roussel. 1982. Pregnancy rates relating to plasma progesterone levels in recipient heifers at day of transfer. Theriogenology 18:365.

Renegar, R. H., H. D. Hafs, J. H. Britt and T. D. Carruthers. 1978. Luteolysis , growth hormone, glucocorticoids, prolactin, and milk production in lactating dairy cows given Prostaglandin F2α. J. Anim. Sci. 47:532.

Rexroad, C. E. and H. D. Guthrie. 1979. Prostaglandin F2α and progesterone release in vitro by ovine luteal tissue during induced luteolysis. Adv. Exp. Med. Biol. 112:639.

Rice, G. E. 1988. Biophysical characteristics of oxytocin secretory granules isolated from ovine corpora lutea. J. Endocrinol. 116:267.

Rice, G. E., G. Jenkin and G. D. Thorburn. 1986. Comparison of particle-associated progesterone and oxytocin in the ovine corpus luteum. J. Endocrinol. 108:109.

Riley, J. C. M. and J. C. Carlson. 1985. Calcium regulated plasma membrane rigidification during corpus luteum regression in the rat. Biol. Reprod. 32:77.

Riley, J. C. M. and J. C. Carlson. 1987. Involvement of phospholipase A activity in the plasma membrane of the rat corpus luteum during luteolysis. Endocrinology 121:776.

Roberts, J. S., B. Barcikowski, L. Wilson, R. C. Skarnes and J. A. McCracken. 1975. Hormonal and related factors affecting the release of prostaglandin F2α from the uterus. J. Steroid Biochem. 6:1091.

Roberts, J.S. and J. A. McCracken. 1976. Does prostaglandin F2α released from the uterus by oxytocin mediate the oxytocic action of oxytocin? Biol. Reprod. 15:457.

Roberts, J. S., J. A. McCracken, J. E. Gavagan and M. S. Soloff. 1976. Oxytocin-stimulated release of prostaglandin F2α from ovine endometrium in vitro: correlation with estrous cycle and oxytocin-receptor binding. Endocrinology 99:1107.

Roche, J. F. 1974. Synchronization of oestrus and fertility following artificial insemination in heifers given prostaglandin F2α. J. Reprod. Fertil. 37:135.

Roche, J. F. 1976. Fertility in cows after treatment with a prostaglandin analogue with or without progesterone. J. Reprod. Fertil. 46:341.

Roche, J. F. and D. J. Prendiville. 1979. Control of estrus in dairy cows with a synthetic analogue of prostaglandin F2 alpha. Theriogenology 11:153.

Rodgers, R. J., J. D. O'Shea, J. K. Findlay, A. P. F. Flint and E. L. Sheldrick. 1983. Large luteal cells the source of luteal oxytocin in the sheep. Endocrinology 113:2302.

Rosenberg, M., Z. Herz, M. Davidson and Y. Folman. 1977. Seasonal variations in post-partum plasma progesterone levels and conception in primiparous and multiparous dairy cows. J. Reprod. Fertil. 51:363.

Rothchild, I. 1981. The regulation of the mammalian corpus luteum. Recent Prog. Horm. Res. 37:183.

Santos, E. 1987. A modified approach for prostaglandin estrous synchronization in cattle. M. S. Thesis. Univ. of Fl., Gainesville.

Saunders, J. O. 1980. History and development of zebu cattle in the United States. J. Anim. Sci. 50:1188.

SAS. 1985. SAS User's Guide: Statistics. Cary, NC: SAS Institute Inc.

Scaramuzzi, R. J., D. T. Baird, H. P. Boyle, R. B. Land and A. G. Wheeler. 1977. The secretion of prostaglandin F from the autotransplanted uterus of the ewe. J. Reprod. Fertil. 49:157.

Schallenberger, E., D. Schams, B. Bullermann and D. L. Waters. 1984. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during prostaglandin-induced regression of the corpus luteum in the cow. J. Reprod. Fertil. 71:493.

Schams, D., A. Lahlou-Kassi and P. Glatzel. 1982. Oxytocin concentrations in two breeds of sheep with high and low fecundity. J. Endocrinol. 92:9.

Schams, D. and H. Karg. 1982. Hormonal responses following treatment with different prostaglandin analogues for estrous cycle regulation in cattle. Theriogenology 17:499.

Schams, D., Th. A. M. Kruip and R. Koll. 1985. Oxytocin determination in steroid producing tissues an in vitro production in ovarian follicles. Acta Endocrinol. 109:530.

Schams, D., S. Prokopp, and D. Barth. 1983. The effect of active and passive immunization against oxytocin on ovarian cyclicity in ewes. Acta Endocrinol. 103:337.

Schams, D., E. Schallenberger, H. H. D. Meyer, B. Bullerman, H. J. Breitinger, G. Enzenhöfer, R. Koll, T. A. M. Kruip, D. L. Walters and H. Karg. 1985. Ovarian oxytocin during the estrous cycle in cattle. In: J. A. Amico and A. G. Robinson (Eds.) Oxytocin: Clinical and Laboratory Studies. pp 317-334. Excerpta Medica, Amsterdam.

Schomberg, D. W. 1967. A demonstration in vitro of luteolytic activity in pig uterine flushings. J. Endocrinol. 38:359.

Schramm, W., L. Bovaird, M. E. Glew, G. Schramm and J. A. McCracken. 1983. Corpus luteum regression concluded by ultra-low pulses of prostaglandin $F2\alpha$. Prostaglandins 26:347.

Schramm, W., N. Eiger-Jensen, G. Schramm and J. A. McCracken. 1986. Local exchange of oxytocin from the ovarian vein to ovarian arteries in sheep. Biol. Reprod. 34:671.

Segerson, E. C., T. R. Hansen, D. W. Libby, R. D. Randel and W. R. Getz. 1984. Ovarian and uterine morphology and function in Angus and Brahman cows. J. Anim. Sci. 59:1026.

Seguin, B. E., D. J. Tate and D. E. Otterby. 1983. Use of cloprostenol in a reproductive management system for dairy cattle. J. Amer. Vet. Med. Assoc. 183:533.

Setchell, B. P. 1977. Male reproductive organs and semen. In: H. H. Cole and P. T. Cupps (Ed.) Reproduction in Domestic Animals (3rd Ed.). p 249. Academic Press, New York.

Sharma, S. C. and R. J. Fitzpatrick. 1974. Effect of oestradiol-17ß and oxytocin treatment on prostaglandin F_α release in the anoestrus ewe. Prostaglandins 6:97.

Sheldrick, E. L. and A. P. F. Flint. 1981. Circulating concentrations of oxytocin during the estrous cycle and early pregnancy in sheep. Prostaglandins 22:631.

Sheldrick, E. L. and A. P. F. Flint. 1985. Endocrine control of uterine oxytocin receptors in the ewe. J. Endocrinol. 106:249.

Sheldrick, E. L., M. D. Mitchell and A. P. F. Flint. 1980. Delayed luteal regression in ewes immunized against oxytocin. J. Reprod. Fertil. 59:37.

Shemesh, M., N. Ayalon and H. R. Lindner. 1972. Oestradiol levels in the peripheral blood of cows during the oestrous cycle. J. Endocrinol. 55:73.

Shemesh, M. and W. Hansel. 1975a. Levels of prostaglandin F (PGF) in bovine endometrium, uterine venous, ovarian arterial and jugular plasma during the estrous cycle. Proc. Soc. Exp. Biol. Med. 148:123.

Shemesh, M. and W. Hansel. 1975b. Stimulation of prostaglandin synthesis in bovine ovarian tissues by arachidonic acid and luteinizing hormone. Biol. Reprod. 13:448.

Shemesh, M. and W. Hansel. 1975c. Arachidonic acid and bovine corpus luteum function. Proc. Soc. Exp. Biol. Med. 148:243.

Short, R. E., R. B. Staigmiller, R. A. Bellows and J. B. Carr. 1978. Breeding cows by AI or natural service at the first or second postsynchronization estrus. J. Anim. Sci. 47 (Suppl. 1):389.

Sirois, J. and J. E. Fortune. 1988. Ultrasonographic monitoring of ovarian follicular dynamics during the estrous cycle in heifers. Theriogenology 29:308.

Smith, R. D., A. J. Pomerantz, W. E. Beal, J. P. McCann, T. E. Pilbeam and W. Hansel. 1984. Insemination of Holstein heifers at a preset time after estrous cycle synchronization using progesterone and prostaglandin. J. Anim. Sci. 58:792.

Soloff, M. S. and M. J. Fields. 1989. Changes in uterine oxytocin receptor concentrations throughout the estrous cycle of the cow. Biol. Reprod. 40:283.

Speroff, L. and R. W. Ramwell. 1970. Prostaglandin stimulation of in vitro progesterone synthesis. J. Clin. Endocrinol. Metab. 30:345.

Spicer, L. J., J. J. Ireland and J. F. Roche. 1981. Changes in serum LH, progesterone and specific binding of ^{125}I-HCG to luteal cells during regression and development of bovine corpora lutea. Biol. Reprod. 25:832.

Spies, H. G., D. R. Zimmerman, H. L. Self and L. E. Casida. 1960. Effect of exogenous progesterone on the corpora lutea of hysterectomized gilts. J. Anim. Sci. 19:101.

Sreenan, J. M. and M. G. Diskin. 1983. Early embryonic mortality in the cow: its relationship with progesterone concentration. Vet. Rec. 112:517.

Stabenfeldt, G. H., L. L. Ewing and L. E. McDonald. 1969. Peripheral plasma progesterone levels during the bovine oestrous cycle. J. Reprod. Fertil. 19:433.

Stabenfeldt, G. H. and J. P. Hughes. 1977. Reproduction in horses. In: H. H. Cole and P. T. Cupps (Ed.) Reproduction in Domestic Animals (3rd Ed.). pp 401-431. Academic Press, New York.

Stacy, B. D., R. T. Gemmel and G. D. Thorburn. 1976. Morphology of the corpus luteum in the sheep during regression induced by prostaglandin F2α. Biol. Reprod. 14:280.

Stellflug, J. N., T. M. Louis, R. C. Gorewit, W. D. Oxender and H. D. Hafs. 1977. Luteolysis induced by prostaglandin F2α before and after hysterectomy in heifers. Biol. Reprod. 17:535.

Stevenson, J. S., M. C. Lucy and E. P. Call. 1987. Failure of timed inseminations and associated luteal function in dairy cattle after two injections of prostaglandin F2-alpha. Theriogenology 28:937.

Stevenson, J. S., M. K. Schmidt and E. P. Call. 1984. Stage of estrous cycle, time of insemination, and seasonal effects on estrus and fertility of Holstein heifers after prostaglandin F2α. J. Dairy Sci. 67:1798.

Stoebel, D. P. and G. P. Moberg. 1982a. Repeated acute stress during the follicular phase and luteinizing hormone surge of dairy heifers. J. Dairy Sci. 65:92.

Stoebel, D. P. and G. P. Moberg. 1982b. Effect of adrenocorticotropin and cortisol on luteinizing hormone surge and estrous behavior in cows. J. Dairy Sci. 65:1016.

Tan, G. J. S., R. Tweedale and J. S. G. Biggs. 1982. Effects of oxytocin on the bovine corpus luteum of early pregnancy. J. Reprod. Fertil. 66:751.

Tanabe, T. Y. and R. C. Hann. 1984. Synchronized estrus and subsequent conception in dairy heifers treated with prostaglandin F2α. I. Influence of stage of cycle at treatment. J. Anim. Sci. 58:805.

Tervit, H. R., L. E. A. Rowson and A. Brand. 1973. Synchronization of oestrus in cattle using a prostaglandin F2α analogue (ICI 79939). J. Reprod. Fertil. 34:179.

Thatcher, W. W., M. Terqui, J. Thimonier and P. Mauleon. 1986. Effect of estradiol 17-ß on peripheral plasma concentration of 15-keto-13,14-dihydro PGF2α and luteolysis in cyclic cattle. Prostaglandins 31:745.

Theodosis, D. T., F. B. P. Wooding, E. L. Sheldrick and A. P. F. Flint. 1986. Ultrastructural localisation of oxytocin and neurophysin in the ovine corpus luteum. Cell Tissue Res. 243:129.

Thomas, J. P., L. J. Dorflinger and H. R. Behrman. 1978. Mechanism of the rapid antigonadotropic action of prostaglandins in cultured luteal cells. Proc. Natl. Acad. Sci. 75:1344.

Thompson, W. J. and S. J. Strada. 1978. Hormonal regulation of cyclic nucleotide phosphodiesterase. In: B. W. O'Malley and L. Birnbaumer (Ed.) Receptors and Hormone Action, Vol. III. pp. 553-557. Academic Press, New York.

Trimberger, G. W. 1948. Breeding efficiency in dairy cattle from artificial insemination at various intervals before and after ovulation. Nebraska Agric. Exp. Stn. Bull. 153:3.

Tritschler, J. P., II, R. T. Duby, R. F. Pekala, A. C. Strong and G. J. Gnatek. 1983. Corpus luteum function in heifers following oxytocin and/or indomethacin treatments. J. Anim. Sci. 57 (Suppl. 1):377.

Tucker, N. P., G. J. Jameson, L. F. Johnston and W. A. North. 1982. Conception rate differences among beef breeds treated with PGF2α. J. Anim. Sci. 55 (Suppl. 1):21.

Turman, E. J., R. P. Wetteman, T. D. Rich and R. Totusek. 1975. Estrous synchronization of range cows with PGF2α. J. Anim. Sci. 41:382 (Abstr.).

Ursely, J. and P. Leymarie. 1979. Varying response to luteinizing hormone of two luteal cell types isolated from bovine corpus luteum. J. Endocrinol. 83:303.

Von Euler, U. S. 1934. Zur kenntnis der pharmakologischen Wirkungen von Nativsekreten und Extrakten maennlicher accessorischer Geschlechtsdruesen. Arch. Exp. Pathol. Pharmacol. 175:78.

Von Euler, U. S. 1935. Ueber die spezifische blutdruk senkende Substanz des menschlichen Prosta- und Samenblasensekretes. Klin. Wochenschr. 14:1182.

Wagner, W. C., R. E. Strohbehn, and P. A. Harris. 1972. ACTH, corticoids and luteal function in heifers. J. Anim. Sci. 35:789.

Wahome, J. N., M. J. Stuart, A. E. Smith, W. R. Hearne and J. W. Fuquay. 1985. Insemination management for a one injection prostaglandin F2α synchronization system. II. One versus two inseminations following detection of estrus. Theriogenology 24:501.

Wakeling, A. E. and L. R. Green. 1981. Corpus luteum prostaglandin receptors and luteolysis. Acta Vet. Scand. 77 (Suppl. 1):131.

Walpole, A. L. 1975. Characteristics of prostaglandins. Ann. Biol. Anim. Biochim. Biophys. 15:389.

Warren, J. E., Jr., P. E. Lewis and D. O. Kiesling. 1979. Inhibition by indomethacin of estrogen-induced luteal regression in heifers. J. Anim. Sci. 49 (Suppl. 1):346.

Waters, R. J. and R. Ball. 1978. Commercial ovulation control and fixed time artificial insemination in cattle. Vet. Record 103:585.

Wathes, D. C. and R. W. Swann. 1982. Is oxytocin an ovarian hormone? Nature 297:225.

Wathes, D. C., R. W. Swann, S. D. Birkett, D. G. Porter and B. T. Pickering. 1983. Characterization of oxytocin, vasopressin, and neurophysin from the bovine corpus luteum. Endocrinology 113:693.

Wathes, D. C., R. W. Swann and B. T. Pickering. 1984. Variations in oxytocin, vasopressin and neurophysin concentrations in the bovine ovary during the oestrous cycle and pregnancy. J. Reprod. Fertil. 71:551.

Watson, J. S., L. F. Archbald and R. A. Godke. 1980. The use of cloprostenol and estradiol cypionate (ECP) to induce luteal regression in intact and hysterectomized beef heifers. J. Anim. Sci. 51 (Suppl. 1):336.

Watts, T. L. and J. W. Fuquay. 1985. Response and fertility of dairy heifers following injection with prostaglandin $F2\alpha$ during early, middle or late diestrus. Theriogenology 23:655.

Webb, R., M. D. Mitchell, J. Falconer and J. S. Robinson. 1981. Temporal relationships between peripheral plasma concentrations of oxytocin, progesterone and 13,14-dihydro-15-keto prostaglandin $F2\alpha$ during the oestrous cycle and early pregnancy in the ewe. Prostaglandins 22:443.

Weber, D. M., P. A. Fields, L. J. Romrell, S. Tumwasorn, B. A. Ball, M. Drost and M. J. Fields. 1987. Functional differences between small and large luteal cells of the late-pregnant vs. nonpregnant cow. Biol. Reprod. 37:685.

Wentz, A. C. and G. S. Jones. 1973. Transient luteolytic effect of prostaglandin $F2\alpha$ in humans. Obstet. Gynec. 42:172.

Wilks, J. W., K. K. Forbes and J. F. Norland. 1972. Synthesis of prostaglandin $F2\alpha$ by the ovary and uterus. J. Reprod. Med. 9:271.

Willet, E. L. 1956. Development in the physiology of reproduction of dairy cattle and artificial insemination J. Dairy Sci. 39:695.

Wiltbank, J. N. and L. E. Casida. 1956. Alteration of ovarian activity by hysterectomy. J. Anim. Sci. 15:134.

Wiltbank, J. N., J. E. Ingalls and W. W. Rowden. 1961. Effects of various forms and levels of estrogens alone or in combinations with gonadotrophins on the estrous cycle of beef heifers. J. Anim. Sci. 20:341.

Wise, T., M. W. Vernon and R. R. Maurer. 1986. Oxytocin, prostaglandins E and F, estradiol, progesterone, sodium and potassium in preovulatory bovine follicles either developed normally or stimulated by follicle stimulating hormone. Theriogenology 26:757.

Wolfenson, D., W. W. Thatcher, M. Drost, D. Caton, D. B. Foster and M. M. LeBlanc. 1985. Characteristics of prostaglandin F measurements in the ovarian circulation during the oestrous cycle and early pregnancy in the cow. J. Reprod. Fertil. 75:491.

Wong, P. Y. and W. Y. Cheung. 1979. Calmodulin stimulates human platelet phospholipase A2. Biochem. Biophys. Res. Comm. 90:473.

Yamauchi, M., T. Nakahara, Y. Kaneda and S. Inui. 1967. Effects of uterine distension on the oestrous cycle of the cow. J. Reprod. Fertil. 13:379.

Zavy, M. T., F. W. Bazer, W. W. Thatcher and C. J. Wilson. 1980. A study of prostaglandin $F2\alpha$ as the luteolysin in swine: V. Comparison of prostaglandin F, progestins, estrone, and estradiol in uterine flushings from pregnant and non-pregnant gilts. Prostaglandins 20:837.

BIOGRAPHICAL SKETCH

Whenever I write one of these things I am tempted to start with that great Dickens' (Dickens, 1980) entry line "I am born." Even though autobiographies are, by definition, of the most personal nature, these imposed sketchs at the end of theses and dissertations are invariably cold and clinical. I can't guarantee this effort will have the warmth and color of a Dickens' composition, but I will endeavor to present you with my past, present and hopes for the future as quickly and painlessly as possible.

Anyway, I was born in Washington, D.C. on the morning of December 27, 1953 to my parents Paul Darke and Eula Mae Cornwell. I am the third in a family of five children. My early education was acquired through attendance in several grammar schools in Maryland and Virginia. When I was 13, we moved to the small town of Ashburn, GA, where I completed junior high and the first three years of high school. In 1971, we relocated to Florida and I graduated from Dunedin High School in 1972.

My collegiate experience began at Central Florida Community College in Ocala. There I was awarded an Associate of Arts degree in July, 1975. I subsequently was accepted for admittance to the University of Florida in August, 1976.

As with many animal science undergraduates, I came to Gainesville under the classification of pre-vet and as a student preparing for a career in veterinary medicine, I was advised to select either animal science or zoology as a major. In truth, I chose animal science because I just couldn't get excited about the rats and drosophila flies in the zoology department. In my first semester at the University of Florida I registered

for the course entitled "Introduction to Animal Science" with a hope of finding out exactly what animal science was. I found my calling. I am convinced there are few professions more honest or noble than those in the field of agriculture.

I finished the required curriculum and received my B.S. degree in June, 1979. I immediately began work towards a Master of Science degree in the same department. My major field of study was ruminant nutrition with an emphasis on the effect of nutrition on reproductive performance in cattle. I received my M.S. in July, 1981. During the course of my research I had the daily responsibility of working with Brahman heifers. These intelligent and unusual animals inspired my admiration and curiosity. When offered an opportunity to stay at UF and conduct further studies in the reproductive physiology of this breed, I accepted.

Work towards my Ph.D. continued through May, 1985. At this time I had completed all research and analysis of data and I accepted a position at Progressive Genetics, an embryo transfer facility. I fully intended to complete the writing of my dissertation while pursuing my new career, but there weren't enough hours in the day. I returned to the University of Florida early in 1988 and expect to complete requirements for my Doctor of Philosophy degree in December, 1989.

Lest you think me only interested in humped cattle, let me add that I love the works of Mozart, Vivaldi, and Willie Nelson. I appreciate the beauty of paintings by Rembrandt, Ruebens, and Rosa Bonhuer (especially her piece <u>Le Labourage nivernais: le Sombrage</u>, owned by the John and Mable Ringling Museum of Art in Sarasota, FL). I like to hike in the mountains, read, and listen to birds. I like quiet evenings at home with my cats. But above all other things, I live to fish.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Michael J. Fields

Michael J. Fields, Chair
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

M Drost

Maartin Drost
Professor of Veterinary Medicine

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James F. Hentges Jr

James F. Hentges, Jr.
Professor Emeritus of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Lynn H. Larkin

Lynn H. Larkin
Professor of Anatomy and Cell Biology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Charles J. Wilcox

Charles J. Wilcox
Professor of Dairy Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1989

Jack L. Fry

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